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PROCEEDINGS OF

THE ROYAL SOCIETY.

Section B.—Biological Sciences.

Trypanosome Diseases of Domestic Animals in Uganda.[*]

II.—*Trypanosoma brucei* (Plimmer and Bradford).

By Colonel Sir David Bruce, C.B., F.R.S., A.M.S.; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, R.A.M.C.; and Captain F. P. Mackie, I.M.S. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10.)

(Received July 15, 1910.)

[Plates 1 and 2.]

Synonym: "Jinja trypanosome," Sleeping Sickness Commission, Royal Society, 1903.

Introduction.

This species was only met with on one occasion during the work of the Commission in 1909. This was in the blood of an ox from the Mabira Rubber Estate (latitude 0° 30′ N., longitude 32° 55′ E.). The manager wrote that the animal came from the Bukedi District, about 100 miles to the north (latitude 1° 50′ N., longitude 32° 40′ E.). Not much is known of this district, as it has only recently come under administration, and therefore it is impossible to say whether the ox was infected in Bukedi or on the journey south.

This is the species of trypanosome which was first discovered by Bruce, in 1894, in Zululand, to be the cause of Nagana, or tsetse-fly disease. During the work of the Sleeping Sickness Commission of the Royal Society in 1903, it was also met with in a herd of cattle from the same district of Bukedi, and then described as the "Jinja trypanosome."[†] It is impossible to name with any certainty the trypanosome seen in 1903, which affected the horses, camels, and dogs of the Abyssinian Boundary Commission. This was described as the "Abyssinian trypanosome." Its morphology, as given in the coloured plate,* shows it to be similar to *Trypanosoma brucei*, so that in all likelihood it was either this species or the closely related *Trypanosoma evansi*. As camels were infected, it was more probably the latter.

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[*] Continued from 'Roy. Soc. Proc.,' B, 1910, vol. 82, p. 479.

[†] 'Reports of the Sleeping Sickness Commission of the Royal Society,' No. VI, p. 112.

In regard to the third kind of trypanosome found in 1903, and referred to as the "Mule trypanosome," no opinion can, at present, be given.

As to the remaining trypanosome seen in 1903, and known as "Pordage's ox trypanosome,"† an examination of the old specimens at once disclosed its identity. It is *Trypanosoma vivax* (Ziemann); and this accounts for the fact that the blood of the ox injected into a monkey and a dog had no effect, these animals being refractory to *Trypanosoma vivax*.

MORPHOLOGY OF TRYPANOSOMA BRUCEI, UGANDA, 1909.

This species is interesting on account of its well-marked dimorphism, in which *Trypanosoma gambiense* resembles it. There are two varieties—the long and slender with free flagellum, and the short and stumpy without free flagellum. The shortest are only 13, and the longest 35 microns in length.

In this paper the long and slender are considered to be those which measure from 25 to 35 microns; the short and stumpy from 13 to 21 microns. Those trypanosomes which measure 22, 23 or 24 microns are called intermediate. The general character of these forms may be given, shortly, as follows:—The long and slender have a narrow extended posterior extremity, an elongated oval nucleus, a well-developed undulating membrane thrown into many waves, folds or plications, and a long free flagellum (see Plate 2, figs. 1 to 5). The short and stumpy, on the other hand, have a rounded posterior extremity, somewhat like the head of a turtle, a round or oval nucleus lying transversely to the long axis of the trypanosome, a well-developed undulating membrane thrown into bold folds, and no free flagellum (see Plate 2, figs. 11 to 16). The intermediate have a short free flagellum, a posterior extremity resembling that of the short and stumpy form but narrower, an oval nucleus, and a well-developed undulating membrane (see Plate 2, figs. 6 to 10).

A. *Living, Unstained.*

No observations were made in Uganda on this species of trypanosome in the living, unstained condition.

B. *Fixed and Stained.*

The blood films were fixed, stained, and measured as described in the 'Proceedings.'‡

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* *Ibid.*, No. VI, plate 2, p. 113.

† *Ibid.*, No. IV, p. 48.

‡ B, 1909, vol. 81, pp. 16 and 17.

Length.—The following table gives the length of this species as found in Uganda, in the ox, monkey, dog, guinea-pig, and rat. Only one trypanosome was found in the blood of the original Mabira ox. It measured 24 microns. In the monkey only 11 trypanosomes could be found. In all the others, 20 trypanosomes have been drawn and measured. These were taken as they came, except that dividing forms were passed by.

Table I.—Measurements of *Trypanosoma brucei*, Uganda strain, 1909.

Date.	No. of expt.	Animal.	Day of disease.	Method of fixing and staining.	In microns.		
					Average length.	Maximum length.	Minimum length.
1909. Aug. 12	1461	Ox	Not known	Osmic acid and Giemsa	24·0	24·0	24·0
Sept. 30	1627	Monkey	26th	„ „	24·8	30·0	19·0
Aug. 26	1479	Dog	41st	„ „	26·5	32·0	22·0
„ 30	1479	„	45th	„ „	22·0	29·0	16·0
Sept. 30	1479	„	76th	„ „	23·5	34·0	20·0
Oct. 11	1644	Guinea-pig	33rd	„ „	20·7	34·0	15·0
„ 18	1644	„	40th	„ „	27·1	34·0	17·0
Sept. 9	1482	Rat	5th	„ „	24·7	30·0	21·0
„ 13	1482	„	9th	„ „	23·5	31·5	19·0
„ 16	1643	„	8th	„ „	19·6	21·0	18·0
					23·6	34·0	15·0

It will be seen from the above table that *Trypanosoma brucei*, Uganda strain, 1909, varies in length between 15 and 34 microns. Doubtless, individuals could be found slightly longer or shorter than this if they were hunted for. In Table I, as already mentioned, the 20 trypanosomes drawn are the first met with, except that dividing forms are left out, and this rule is adhered to throughout.

The average length of the 172 trypanosomes dealt with in Table I is 23·6 microns; but, as this does not give much information, the following table has been prepared. This represents by means of dots the length of each of 160 trypanosomes, in eight preparations of 20 each. Although *Trypanosoma brucei* is described as a dimorphic species, it must not be supposed that there is any strict line of demarcation between the long and slender and short and stumpy forms. There are the intermediate forms. This table is also useful in showing at a glance the distribution of the various lengths.

Table II.—Represents the Distribution in respect to Length of *Trypanosoma brucei*, Uganda strain, 1909.

Expt. No.	Short and stumpy.							Intermediate.			Long and slender.											Average, in microns.
	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	
1479								●●●		●	●●	●●●●	●●	●●●●	●	●●		●				26·5
1479		●	●		●	●●●	●●	●●	●●●●	●●●				●	●●							22·0
1479						●●●●	●●●●	●●●●		●	●●●					●			●●	●		23·5
1644	●	●	●●	●●●●	●	●	●●●	●●	●●	●				●						●		20·7
1644			●		●	●					●●●	●	●●	●	●●●●	●●●	●		●	●		27·1
1482							●	●●	●●●●●	●●●	●●●	●	●	●●	●	●						24·7

1482					●●●	●●●●	●●●	●	●		●		●	●●●	●●			●				23·5
1643				●●	●●●●	●●●●●●●●●●●●●	●															19·6
Totals......	1	2	4	6	10	26	14	14	12	9	12	6	6	12	10	7	1	2	3	3		
Per- centage }	0·6	1·3	2·5	3·8	6·2	16·2	8·7	8·7	7·5	5·6	7·5	3·8	3·8	7·5	6·2	4·4	0·6	1·3	1·9	1·9		

This division of *Trypanosoma brucei* into long, intermediate, and short forms is, of course, quite artificial, as the one form passes into the other by insensible gradations. It may, however, prove of some use in the identification of this species. In the same way a series of measurements of the various parts of the trypanosome body may also prove useful. The following table gives the distance between the posterior extremity and the micronucleus, the micronucleus and the nucleus, the size of the nucleus, the distance between the nucleus and the anterior extremity, and the length of the free flagellum.

Breadth.—The long and slender forms average 1·5 micron; the short and stumpy 2·5 microns.

Shape.—As already mentioned, this is a markedly dimorphic species, composed of long, slender forms with free flagella, and short, stout forms without free flagella. It is curious that the long forms may preponderate in the blood one day and the short forms another. For example, as will be seen from Table I, the trypanosomes in the guinea-pig's blood on October 11 were mostly short forms, giving an average of 20·7 microns, while a week later they were mostly long forms, giving an average of 27·1 microns. Note also Rat 1643, with an average of 19·6 microns, and Rat 1482, with an average of 24·7 microns.

This species may be compared with Dutton and Todd's *Trypanosoma dimorphon*, and in truth the coloured drawings given by them in their original plate* (figs. VII, IX, and X) are very similar, if not identical, in shape and size with *Trypanosoma brucei*. Fig. X, the long, slender form with free flagellum, is 30·5 microns in length. Fig. VII, the short, stumpy form without free flagellum, is 16 microns in length. As to fig. VI, on the same plate, stated to represent the "tadpole form," one would suspect from its general shape and appearance that it had no connection with the others. But in regard to figs. VII and X, anyone comparing Dutton and Todd's plate with the coloured plate accompanying this paper must be struck with the close resemblance between these two forms of *Trypanosoma dimorphon* and *Trypanosoma brucei*. Is it possible that Dutton and Todd were dealing with *Trypanosoma brucei* when they described *Trypanosoma dimorphon*?

Contents of Cell.—The protoplasm which is stained a pale blue is often dotted over with chromatin granules, especially in the anterior half (Plate 2). In well-stained preparations the distribution and number of these granules is sufficient, according to McFadyean, to differentiate this species from *Trypanosoma evansi*. This was also pointed out by Laveran

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* 'First Report of the Trypanosomiasis Expedition to Senegambia (1902),' University Press of Liverpool, 1903.

Table III.—Measurements of the various Parts of *Trypanosoma brucei*, Uganda strain, 1909.

Experiment.	Posterior extremity to micronucleus.	Micronucleus to nucleus.	Nucleus.	Nucleus to anterior extremity.	Free flagellum.	Total length.
			Long and Slender Forms.			
1644	4	5	3·5	11·5	10	34
1644	3	7	4	10	10	34
1644	3	6·5	3·5	13	7	33
1644	2·5	7·5	3	10	8	31
1644	2	7	3·5	12·5	5	30
1644	2	7	3	11	7	30
1644	2	7	3	8	10	30
1482	1	7	3	11	8	30
1627	2	6	2·5	10·5	9	30
1627	3	7·5	2·5	13	4	30
1644	2	6	3	12	6	29
1644	3·5	5·5	3·5	9·5	7	29
1644	2	6·5	3	12·5	5	29
1644	2·5	7	3	6·5	10	29
1482	2	6	3·5	9·5	8	29
1644	3	6	3	8	8	28
1644	2	7	3	16	—	28
1482	1·5	6·5	2·5	13·5	4	28
1482	1·5	8	2·5	8	8	28
1627	1	7·5	2·5	11	6	28
1627	1	7·5	2·5	8	9	28
1644	2	7	3	10	5	27
1644	2	6	3·5	8·5	7	27
1627	1	7	3	9	7	27
1482	2	7·5	2	11·5	4	27
1644	2	6	2·5	10·5	5	26
1482	2	7	3	9·5	4·5	26
1644	2	6	3	7	7	25
1644	2	7	3·5	8·5	4	25
1644	1·5	5·5	3	10	5	25
1482	2	5·5	2·5	10	5	25
1482	1	6	3	11	4	25
1482	2	6·5	3	8·5	5	25
1627	2	6	2·5	8·5	6	25
Average...	2·1	6·6	3·0	10·2	6·4	—
			Intermediate Forms.			
1644	1	6	2·5	14·5	—	24
1482	2	6·5	3·5	8	4	24
1482	1	6	3	10	4	24
1482	1·5	6	3	9·5	4	24
1644	1	5	2·5	14·5	—	23
1644	1	7·5	2·5	12	—	23
1482	1	6	3	8·5	4·5	23
1482	2	5	2·5	8·5	5	23
1482	2	5	4	9	3	23
1482	1	5·5	3	9	4·5	23
1482	2	5	3	10	3	23
1627	1·5	7	3·5	4	7	23
1644	2	6·5	3	5·5	5	22
1644	1	7	3·5	6·5	4	22
1482	2	5·5	2	12·5	—	22
1482	1·5	6	2·5	7	5	22
1627	1	6	2	7	6	22
Average...	1·4	6·0	2·9	9·2	3·5	—

Table III—*continued.*

Experiment.	Posterior extremity to micronucleus.	Micronucleus to nucleus.	Nucleus.	Nucleus to anterior extremity.	Free flagellum.	Total length.
Short and Stumpy Forms.						
1644	1	6	3	11	—	21
1644	1	5·5	2·5	12	—	21
1644	1	5·5	2·5	12	—	21
1482	2	5	3	11	—	21
1643	1	4·5	2·5	13	—	21
1627	1·5	6·5	3	7·5	2·5	21
1644	2	3	3	12	—	20
1644	1	5	2	12	—	20
1643	1	4·5	2	12·5	—	20
1643	1	4	2·5	12·5	—	20
1643	1	6	2·5	10·5	—	20
1643	1	5	2·5	11·5	—	20
1643	1	5·5	2	11·5	—	20
1643	1	5	3	11	—	20
1643	1	5·5	2·5	11	—	20
1643	1	4·5	2·5	12	—	20
1643	1	4	2·5	12·5	—	20
1643	1·5	5·5	2	11	—	20
1643	1	4	2·5	12·5	—	20
1643	1	5	2	12	—	20
1643	1	4·5	2	12·5	—	20
1627	1	6	2	11	—	20
1644	1	5	2	11	—	19
1644	1	3·5	2	12·5	—	19
1643	1	5	2·5	10·5	—	19
1643	1	5	2	11	—	19
1643	1	4·5	2	10·5	—	19
1643	1	5·5	3	10·5	—	19
1627	1	7	3	8	—	19
1644	1	4	2·5	10·5	—	18
1644	1	4	2·5	10·5	—	18
1644	1	3·5	3	10·5	—	18
1644	1	6	2·5	8·5	—	18
1643	1	4·5	2·5	10	—	18
1643	2	4	2·5	9·5	—	18
1644	1	5	3	8	—	17
1644	1	4·5	2·5	9	—	17
1644	1	6	2·5	7·5	—	17
1644	1	5·5	2	7·5	—	16
1644	1	5	2	7	—	15
Average ...	1·1	4·9	2·4	10·7	—	—
Average of three forms	1·5	5·8	2·8	10·0	3·3	—

and Mesnil some years ago, when they wrote: "Avec un peu d'habitude on arrive au contraire à distinguer les deux Trypanosomes, quand on dispose de préparations bien colorées et riches en parasites."*

Nucleus.—Is oval or elongated in shape in the long forms, a short oval in the intermediate, round or oval in the short, stumpy forms, and situated about the middle of the body.

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* 'Trypanosomes et Trypanosomiases,' 1904.

Micronucleus.—Small and round, situated, on an average, 2·1 microns from the posterior extremity in the long and slender forms, 1·4 microns in the intermediate, and 1·1 microns in the short and stumpy.

Undulating Membrane.—This is well developed in this species and thrown into many folds and undulations. In this it differs markedly from *Trypanosoma vivax.*

Flagellum.—The flagellum in the long and slender forms is free and, on an average, 6·4 microns in length. In the short and stumpy forms there is no free flagellum.

COMPARISON OF TRYPANOSOMA BRUCEI, UGANDA, 1909, WITH TRYPANOSOMA BRUCEI, ZULULAND, 1894 (see Plate 1).

When *Trypanosoma brucei* was discovered in Zululand, in 1894, it was naturally thought to be the one and only trypanosome in Africa, and a detailed description seemed unnecessary. Now, however, since the number of species in Africa has increased to such an alarming extent, it is necessary to add to the old description by more detailed measurements. Luckily, many of the old Zululand preparations are still extant, so that it has been possible to do this. It must, however, be borne in mind that the Zululand preparations are some 15 years old, and were stained with carbol-fuchsin and not by Giemsa as in the case of the Uganda strain. They may, therefore, have shrunk to a greater extent than the more recently stained specimens.

Table IV.—Measurements of the Original Strain of *Trypanosoma brucei,* discovered in Zululand in 1894.

Trypanosoma brucei, Zululand, 1894.

Date.	No. of expt.	Animal.	Method of fixing and staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
1/11/95	212	Horse	Osmic acid; carbol-fuchsin	22·4	32·0	14·5
1/11/95	212	,,	,, ,,	20·6	32·5	14·0
7/11/95	219	Donkey	,, ,,	23·0	35·0	14·5
23/11/95	214	Ox	,, ,,	18·0	25·5	13·0
4/7/97	—	Monkey	,, ,,	20·5	32·0	15·0
6/1/95	190	Dog	,, ,,	25·0	35·0	17·0
10/12/95	—	,,	,, ,,	24·0	32·0	17·0
14/1/96	233	,,	,, ,,	27·8	34·0	18·0
25/1/96	244	,,	,, ,,	20·6	33·0	15·0
7/2/96	229	,,	,, ,,	28·7	35·0	19·0
9/7/97	433	,,	,, ,,	19·7	29·0	17·0
				22·8	35·0	13·0

Table V.—Represents the Distribution in respect to Length of *Trypanosoma brucei*, Zululand strain, 1894.

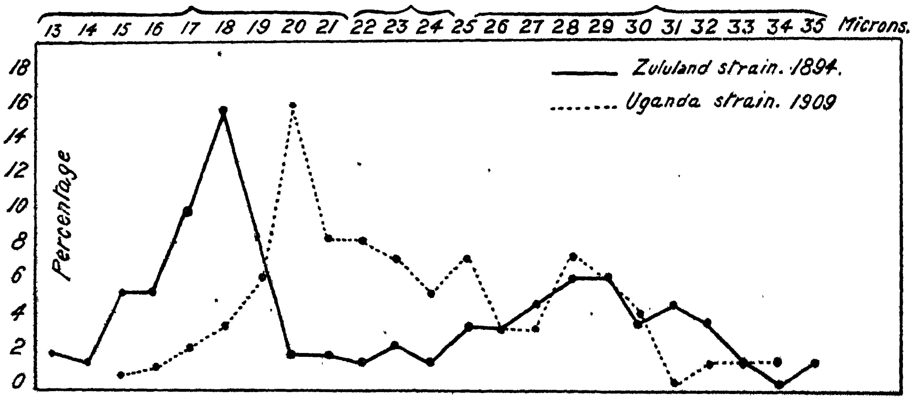
Expt. No.	Short and stumpy.									Intermediate.			Long and slender.											Average, in microns.
	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	
212			●	●●	●●	●●●	●			●	●			●●	●	●	●●	●●		●				22·4
212		●	●●●	●●	●●	●●●●	●						●●	●●	●●					●				20·6
219			●●●	●●●	●	●	●						●	●	●●	●●	●●●		●				●	23·0
214	●●●	●●	●	●	●●●●●	●		●	●●●	●	●		●											18·0
—	●		●●	●●	●●●●●	●	●●					●	●		●	●	●	●		●				20·5
—					●●	●●●	●	●	●	●	●●			●	●	●	●	●●	●●	●				24·0
233						●●	●				●		●		●●●	●●	●	●	●●●	●●	●●	●		27·8

244			●	●		●●●●●●●	●●●●●	●●								●	●	●			●			20·6
229							●●					●	●			●●●●	●●●	●	●●●●	●●			●●	28·7
433					●●●	●●●●●●●●●●	●●●					●		●		●	●							19·7
Totals…………	4	3	11	11	20	32	17	4	4	3	5	3	7	7	10	13	13	8	10	8	3	1	3	
Percentage……	2	1·5	5·5	5·5	10	16	8·5	2	2	1·5	2·5	1·5	3·5	3·5	5	6·5	6·5	4	5	4	1·5	0·5	1·5	

The average length of the *Trypanosoma brucei*, Uganda, 1909, was 23·6 microns, the maximum 34 microns, and the minimum 15 microns. The measurements of the two strains therefore correspond very closely.

Tables II and V can be compared in the following chart, which gives the curves of the distribution in respect to length of *Trypanosoma brucei*, Uganda, 1909, and *Trypanosoma brucei*, Zululand, 1894.

Chart giving Curves of the Distribution, by percentages, in respect of Length, made up from Tables II and V.



From these curves it will be seen that there is a marked resemblance between the two strains when represented in this way.

Animals Susceptible to Trypanosoma brucei.

Table VI shows the effect of inoculating various animals with *Trypanosoma brucei*, Uganda strain, 1909.

The number of experiments carried out on the various animals is too small for comparative purposes, but sufficient to show that cattle and the smaller laboratory animals are susceptible to this trypanosome.

Cultivation of Trypanosoma brucei, Uganda Strain, 1909.

No attempt was made in Uganda to cultivate this trypanosome.

Carrier of Trypanosoma brucei.

No experiments were made in the laboratory at Mpumu with *Glossina palpalis* as a carrier of this trypanosome, and no evidence is to hand as to how it is conveyed from sick to healthy animals in the district of Bukedi.

Table VI.

Date.	Expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.	Remarks.
			Cattle.		
1909. Aug. 11 ...	1461	Nat. infec.	?	—	Killed on 79th day.
			Goat.		
Aug. 16 ...	1478	Ox 1461	—	—	Never showed trypanosomes; under observation 37 days.
			Monkey.		
Aug. 16 ...	1480	Ox 1461	—	—	Died 10 days after inoculation; never showed trypanosomes.
Sept. 4 ...	1627	Dog 1479	13	69	Died of *T. brucei.*
			Dog.		
Aug. 16 ...	1479	Ox 1461	8	49	Died of *T. brucei.*
			Rabbit.		
Sept. 8 ...	1645	Rat 1482	26	—	Still alive after 67 days.
			Guinea-pig.		
Aug. 16 ...	1481	Ox 1461	—	—	Never showed trypanosomes; under observation 18 days.
Sept. 3 ...	1481	Dog 1479	5	—	Still alive after 71 days.
„ 8 ...	1644	Rat 1482	26	—	„ „ 67 „
			Rat.		
Aug. 16 ...	1482	Ox 1461	—	—	Never showed trypanosomes; under observation 18 days.
Sept. 4 ...	1482	Dog 1479	5	23	Died of *T. brucei.*
„ 8 ...	1643	Rat 1482	8	22	„ „
			Mouse.		
Aug. 16 ...	1483	Ox 1461	—	—	Accidentally killed on 9th day; never showed trypanosomes.

CONCLUSION.

With the evidence available, the Commission consider themselves justified in considering the trypanosome recovered from the Uganda ox to be identical with *Trypanosoma brucei*, the cause of Nagana in Zululand and other parts of South Africa.

DESCRIPTION OF PLATES.

PLATE 1.

(1) *Trypanosoma brucei*, Zululand, 1894, stained carbol-fuchsin. × 2000.

Figs. 1 and 2.—Long and slender forms.
Figs. 3 and 4.—Intermediate forms.
Figs. 5, 6 and 7.—Short and stumpy forms.

(2) *Trypanosoma brucei*, Uganda, 1909, stained Giemsa. × 2000.

Figs. 8 and 9.—Long and slender forms.
Fig. 10.—Intermediate form.
Figs. 11, 12 and 13.—Short and stumpy forms.

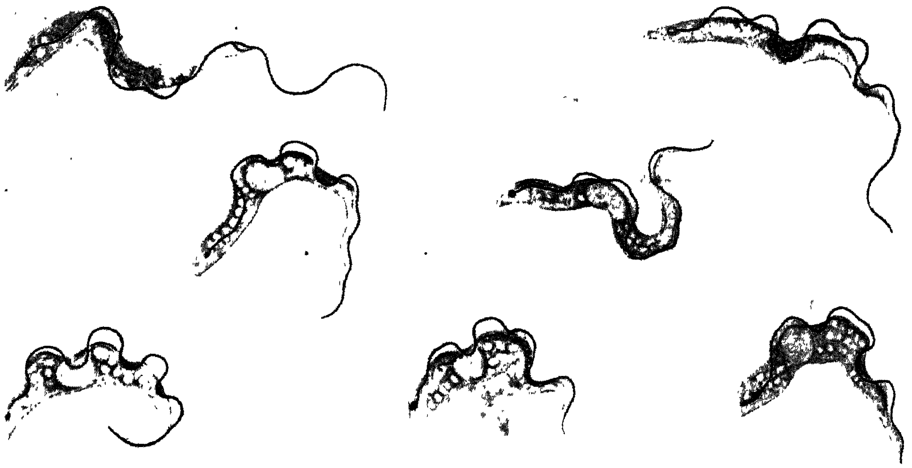
(3) *Trypanosoma dimorphon* (?), Khartoum. × 2000.

Figs. 14 and 15.—Long and slender forms.
Fig. 16.—Intermediate form.
Figs. 17, 18 and 19.—Short and stumpy forms.

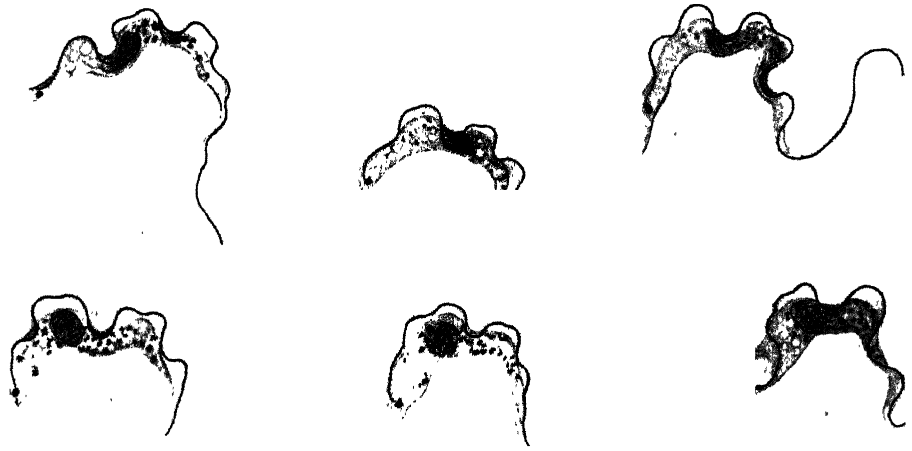
PLATE 2.

Trypanosoma brucei, Uganda, 1909, stained Giemsa. × 2000

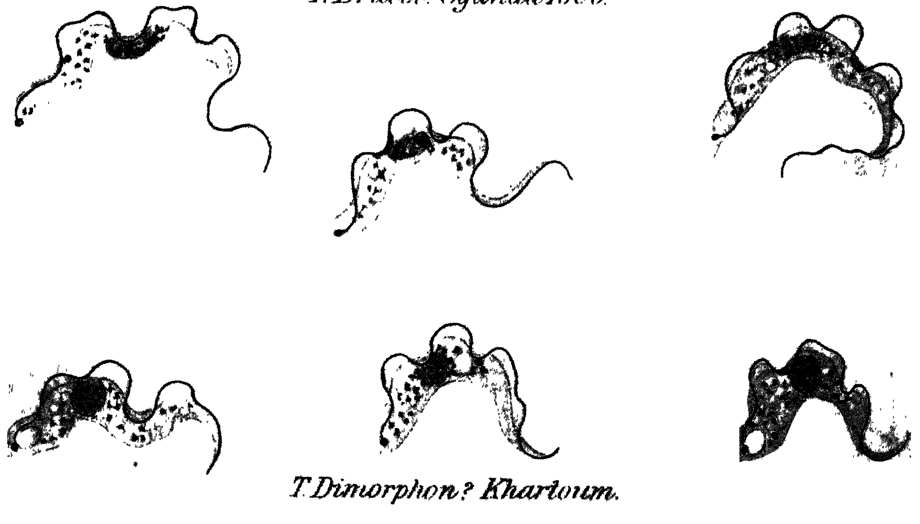
Figs. 1–5.—Long and slender forms.
Figs. 6–10.—Intermediate forms.
Figs. 11–16.—Short and stumpy forms.



T. Brucei. Zululand 1894.



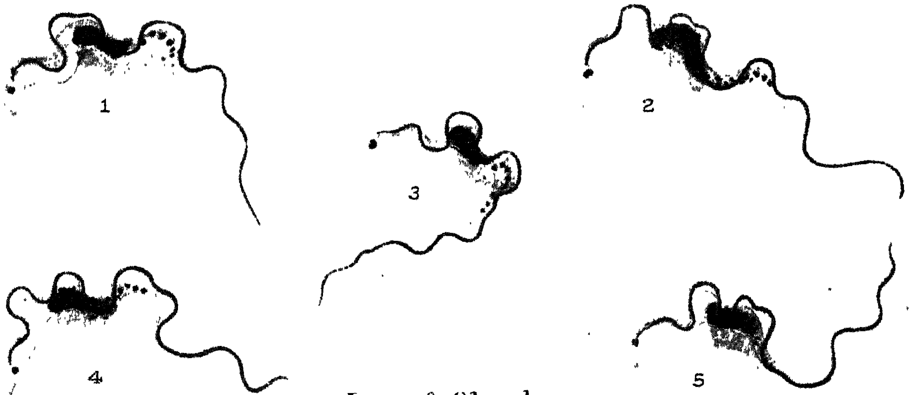
T. Brucei. Uganda 1909.



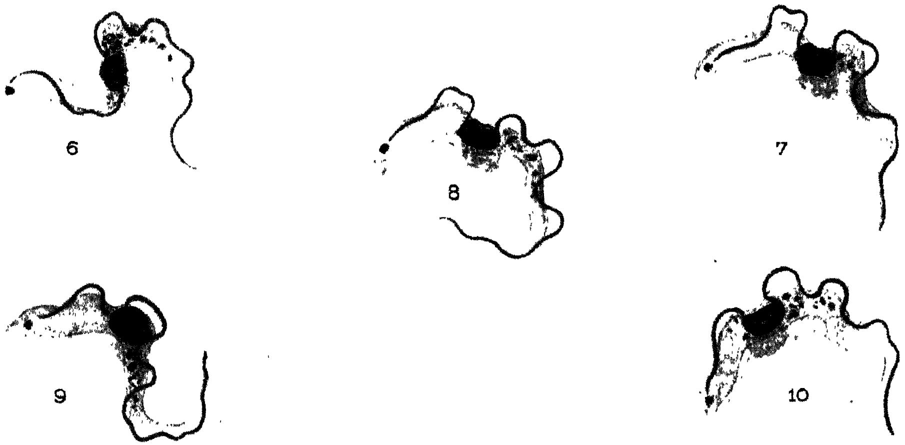
T. Dimorphon? Khartoum.

M. E. Bruce, del.

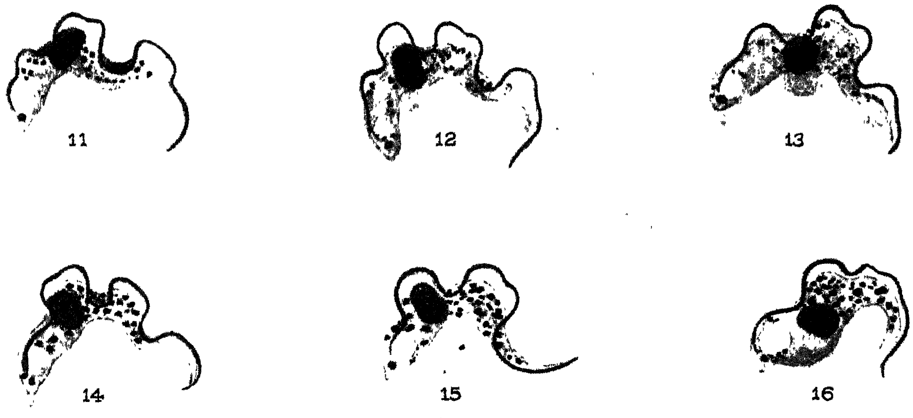
Bruce & others Roy. Soc. Proc B. vol. 83, Pl 2



Long & Slender.



Intermediate.



Short & Stumpy.

M. E. Bruce, del.

WATERLOW & SONS LIMITED, LONDON WALL, LONDON

*Trypanosome Diseases of Domestic Animals in Uganda.**

III.—*Trypanosoma vivax* (Ziemann).

By Colonel Sir David Bruce, C.B., F.R.S., A.M.S.; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, R.A.M.C.; and Captain F. P. Mackie, I.M.S. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10.)

(Received July 22, 1910.)

[Plates 3—5.]

Synonyms.
- Trypanosome of Pordage's ox, 1903 (Sleeping Sickness Commission of the Royal Society, 1903).
- *Trypanosoma cazalboui* (souma). Laveran.

Introduction.

This interesting species of trypanosome appears to be widely distributed in Uganda. It was first discovered by the Commission in two cattle which came from Kavirondo, the district lying to the north-east of Victoria Nyanza. These oxen were driven to Kampala round the north end of the Lake, and probably became infected on the way.

Then the Government Transport Department lost many of their oxen from this trypanosome. They were worked between Kampala, the native capital, and Luzira, the port on the Lake-shore, which lies about seven miles to the south-east. When the epidemic broke out these cattle were kraaled near the Lake-shore, along which they were allowed to graze, and where tsetse-flies are numerous. Afterwards, at the suggestion of the Commission, they were kraaled at Kampala, when the epidemic stopped, and no more deaths from *Trypanosoma vivax* occurred among them.

Cases also occurred among the transport cattle belonging to the German Company, "Victoria Nyanza Agentur." These cattle were employed carrying goods between Lake Victoria and Lake Albert.

Lastly, the *Glossina palpalis* on the Lake-shore near Mpumu, where the Sleeping Sickness Commission laboratory was placed, were found to be naturally infected with *Trypanosoma vivax*, and this trypanosome was found in the blood of a bush-buck, shot at the same place at which the flies were collected.

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* Continued from preceding article.

The evidence, therefore, at present points to the *Glossina palpalis* being the carrier of this disease, and that the wild animals living in the fly area act as a reservoir of the virus.

This species of trypanosome is similar to *Trypanosoma nanum*, in that it is only pathogenic to equines and bovines, and has no effect on the smaller laboratory animals. There was no opportunity in Uganda of inoculating it into horses, mules, or donkeys.

Nine cattle came under observation suffering from a natural infection of the disease. Seven of these died after being at Mpumu, on an average, 103 days. Five cattle were successfully inoculated, and these died, on an average, in 69 days. The disease is, therefore, a fairly rapid and fatal one in cattle. It may be noted that two of the cattle (Experiments 493 and 583), after living for 268 and 237 days respectively, died at last from an accidental infection of *Trypanosoma pecorum*. It is possible that these two oxen had recovered, and if not, it is certain that trypanosomes were either absent or very scanty in their blood, as inoculations from them failed to infect goats and sheep. It may also be noted that none of the four sheep which were inoculated ever showed signs of the disease. This is probably due to the scarcity or absence of the trypanosomes in the blood used in the experiment for inoculation, and not to any natural immunity.

MORPHOLOGY OF TRYPANOSOMA VIVAX.

This species of pathogenic trypanosome can at once be recognised among all the others by its shape alone. There is no tendency to dimorphism, as in *Trypanosoma brucei* and *Trypanosoma gambiense*. On the contrary, the individuals tend to run persistently to one type. This type has an average length of 24 microns. The body posterior to the nucleus is swollen, and contains clear protoplasm, in which an alveolar structure can be made out; the posterior end is rounded, as a rule, and close to it is the large round micronucleus; the anterior part of the body rapidly narrows and terminates in a free flagellum; the nucleus is elongated and situated in the narrowed part, is often broken up and diffused, and seems to fill up the part of the body in which it lies; the undulating membrane is narrow and simple, and can, as a rule, only be made out by the deeply-stained flagellum running along its border. An examination of the plates which illustrate this paper will, however, give a clearer idea of the general appearance of this species than any written description.

A. *Living, Unstained.*

This species of trypanosome is extremely active in its movements. It dashes across the field of the microscope with such rapidity that it is impossible to follow its movements, cyclone-like leaving a clear path, the corpuscles in its track having been flung on either side. If it remains at the same spot for a time, as it sometimes does, it has an appearance of great energy and power, throwing the surrounding red blood corpuscles about in wild confusion.

B. *Fixed and Stained.*

The blood-films were fixed, stained and measured as described in the 'Proceedings.'*

Length.—The following table gives the average length of this trypanosome in the ox and goat. Twenty trypanosomes are drawn and measured from each preparation, the average length reckoned, and the length of the longest and shortest given.

Table I.—*Trypanosoma vivax*, Uganda, 1909.

No of experiment.	Animal.	Method of fixing.	Method of staining.	In microns		
				Average length.	Maximum length	Minimum length.
290	Ox	Osmic acid	Giemsa	23·7	28·0	21·0
445	"	"	"	23·8	26·0	20·0
450	"	"	"	23·0	25·0	21·0
450	"	"	"	23·4	25·0	22·0
493	"	"	"	23·7	25·0	21·0
597	"	"	"	25·1	28·0	22·0
598	"	"	"	25·8	28·0	24·0
779	"	"	"	24·6	27·0	21·0
982	"	"	"	23·5	25·0	22·0
1267	"	"	"	24·2	27·0	20·0
1462	"	"	"	23·8	28·0	16·0
864	Goat	"	"	24·2	25·0	23·0
864	"	"	"	24·7	26·0	19·0
1036	"	"	"	23·5	29·0	19·0
				24·1	29·0	16·0

As is shown by the above table, this trypanosome varies in length between 16 and 29 microns. Individuals only 16 microns long are rare, and only occur immediately after division. The following table represents the distribution of length among 180 trypanosomes, and it will be seen by it that only three are found to measure less than 19 microns.

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* B, 1909, vol 81, pp. 16, 17.

Table II.—Represents the Distribution in respect to Length of *Trypanosoma vivax*, Uganda strain, 1909.

Expt. No.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	Average, in microns.
445								●		●●	●●●	●●●●●●●	●●●●●●	●										23·8
450							●●	●●●●	●●●●●●●	●●●●●●	●													23·0
597										●	●	●●●	●●●●●●●●●	●●●	●●	●								25·1
598												●●●●	●	●●●●●●●●●●●	●●●	●								25·8

779									●	●●	●●	●●	●●●●●●●	●●●	●●●									24·6
1267								●	●		●●●●●	●●●	●●●●	●●●●●	●									24·2
1462				●	●●					●	●	●●●●●	●●●	●●●●	●●	●								23·8
864							●				●●	●●	●●●●●●●●●	●●●●●●										24·7
1036							●	●●	●	●●●●	●●●	●	●●●●●	●			●●							23·5
Totals…………	—	—	—	1	2	—	4	8	10	16	18	27	44	34	11	3	2	—	—	—	—	—	—	
Percentage……	—	—	—	0·6	1·1	—	2·3	4·4	5·5	8·9	10	15	24·4	18·9	6·1	1·7	1·1	—	—	—	—	—	—	

Breadth.—Varies between 2 and 3 microns.

Shape.—This species can be recognised with certainty by its shape alone. The body of the creature lies mostly posterior to the nucleus, and this part is broad and swollen and filled with beautifully clear protoplasm, in which a delicate alveolar structure can be made out. The body narrows at the nucleus and tapers off rapidly to the anterior extremity (Plates 3 and 4).

Contents of Cell.—Clear, with a delicate alveolar structure, and now and then a chromatin-staining granule, especially in the narrow anterior part.

Nucleus.—Long and oval, often diffuse, situated towards the anterior extremity, and in a narrowed or waist-like part of the trypanosome.

Micronucleus.—Large, round and terminal, or sub-terminal.

Undulating Membrane.—Narrow, simple, straight, and little in evidence.

Flagellum.—There is a well-marked flagellum, the free part varying from 3 to 6 microns in length (Plates 3 and 4).

COMPARISON OF TRYPANOSOMA VIVAX, UGANDA, 1909, WITH TRYPANOSOMA VIVAX, TOGOLAND, 1903.

Thanks to the courtesy of the Director of the Hamburg Institute of Tropical Medicine, through Dr. Fülleborn, the Commission are enabled to compare the Uganda strain with the original preparations made by Ziemann in 1903.

Table III.—Measurements of the Original Strain of *Trypanosoma vivax* (Ziemann), 1903.

Animal.	Method of fixing and staining.	In microns.		
		Average length.	Maximum length.	Minimum length.
Ox	?	24 ·8	26 ·0	23 ·0
„	?	26 ·5	31 ·0	23 ·0
Sheep	?	21 ·4	23 ·0	18 ·0
„	?	22 ·1	25 ·0	20 ·0
	Average......	23 ·7	31 ·0	18 ·0

The trypanosomes found in the blood of this sheep were shorter, narrower, and have a more pointed posterior extremity that in the type described. Whether this is the rule or only exceptional it is impossible with our scanty material to say.

Table IV.—Represents the Distribution in respect to Length of *Trypanosoma vivax*, Togoland strain, 1903.

Expt. No.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	Average, in microns.
Ox											●●●	●●●●	●●●●●●●	●●●●●●										24·8
Ox											●	●	●●●	●●●	●●●●●●●●●	●●			●					26·5
Sheep						●	●●	●	●●●●●	●●●●●●	●●●●●													21·4
Sheep								●●●●	●●●	●●●●	●●●●●	●●●	●											22·1
Totals	—	—	—	—	—	1	2	5	8	10	14	8	11	9	9	2	—	—	1	—	—	—	—	
Percentage	—	—	—	—	—	1·2	2·5	6·3	10	12·5	17·5	10	13·7	11·3	11·3	2·5	—	—	1·2	—	—	—	—	

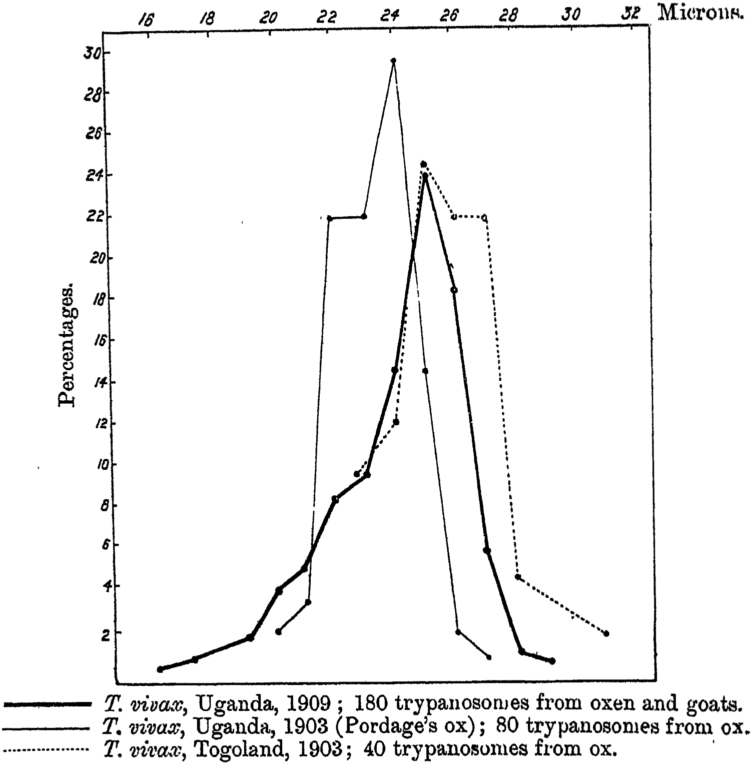
COMPARISON OF TRYPANOSOMA VIVAX, UGANDA, 1909, WITH TRYPANOSOMA VIVAX, 1903 (PORDAGE'S OX).

On examining the preparations made in 1903 from two oxen belonging to the Uganda Transport, the trypanosomes in them were at once recognised as belonging to this species (Plate 3).

Table V.—Measurements of *Trypanosoma vivax*, Uganda, 1903 (Pordage's Ox).

No. of experiment.	Animal.	Method of fixing.	Method of staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
1	Ox	Osmic acid	Leishman	23·3	25·0	21·0
1	”	”	”	23·1	26·0	20·0
2	”	”	”	23·3	25·0	22·0
2	”	”	”	23·5	27·0	20·0
			Average...	23·3	27·0	20·0

CHART giving curves representing the distribution, by percentages, in respect of length of *Trypanosoma vivax*, Uganda, 1909, *Trypanosoma vivax*, Uganda, 1903 (Pordage's ox), and *Trypanosoma vivax*, Togoland, 1903.



━━━━ *T. vivax*, Uganda, 1909; 180 trypanosomes from oxen and goats.
──── *T. vivax*, Uganda, 1903 (Pordage's ox); 80 trypanosomes from ox.
·········· *T. vivax*, Togoland, 1903; 40 trypanosomes from ox.

Table VI.—Represents the Distribution in respect to Length of *Trypanosoma vivax*, Uganda, 1903 (Pordage's Ox).

Expt. No.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	Average, in microns.
1									●	●●●●	●●●●●●	●●●●●	●●●●											23·3
1								●		●●●●●●	●●●●●	●●●●●	●●	●										23·1
2										●●●●●●	●●●●	●●●●●●●	●●●											23·3
2								●	●●	●●	●●●	●●●●●●●	●●●	●	●									23·5
Totals	—	—	—	—	—	—	—	2	3	18	18	24	12	2	1	—	—	—	—	—	—	—	—	
Percentage	—	—	—	—	—	—	—	2·5	3·7	22·5	22·5	30	15	2·5	1·3	—	—	—	—	—	—	—	—	

From a comparison of these tables and the figures on Plate 3, there can be little doubt that the trypanosome found in the blood of cattle in Uganda in 1909 is identical with that seen in 1903, and also with that discovered by Ziemann in Togoland, and named by him *Trypanosoma vivax.*

As Dr. Laveran kindly examined the Uganda specimens and pronounced them to be *Trypanosoma cazalboui,* it is probable that *Trypanosoma vivax* and *Trypanosoma cazalboui* are the same species.

ANIMALS SUSCEPTIBLE TO TRYPANOSOMA VIVAX.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.	Remarks.
			Cattle.		
1909.					
July 12	431	Nat. infec.	?	—	Alive after 38 days.
Jan. 14	450	,,	?	48	Died of *T. vivax.*
Feb. 2	493	,,	?	268	Also infected by *T. pecorum.*
Mar. 6	583	,,	?	237	,, ,,
,, 14	597	,,	?	49	Died of *T. vivax.*
,, 14	598	,,	?	79	,, ,,
May 16	779	,,	?	6	,, ,,
July 12	1309	,,	?	40	,, ,,
,, 12	1318	,,	?	—	Alive after 30 days.
Sept. 29	290	Ox 1465	30	32	Died of *T. vivax.*
Mar. 3	467	Ox 450	—	—	Experiment stopped after 77 days.
May 19	869	Oxen 493, 583, & 598	—	—	,, ,, 101 ,,
,, 19	870	,,	—	—	,, ,, 101 ,,
July 27	737	Goat 864	15	60	Died of *T. vivax.*
June 21	1030	,,	10	83	,, ,,
July 6	1267	,,	22	55	,, ,,
,, 6	1268	,,	15	114	Killed.
		Average ...	18	89	
			Goat.		
Mar. 31	623	Ox 598	—	—	Experiment stopped after 48 days.
Apr. 2	636	Ox 494	—	—	,, ,, 46 ,,
,, 3	639	Ox 583	—	—	,, ,, 45 ,,
May 19	864	Ox 779	15	148	Died of *T. vivax.*
Aug. 4	1419	Ox 1309	22	—	Still alive after 30 days.
Sept. 10	1652	Ox 1318	21	31	Died of *T. vivax.*
June 22	1036	Goat 864	27	29	,, ,,
—	1037	,,	—	—	Never showed trypanosomes; under observation 66 days.
June 25	1079	,,	21	—	Experiment stopped after 21 days.
July 19	1344	,,	36	—	,, ,, 36 ,,
,, 26	1383	,,	24	—	,, ,, 24 ,,
Aug. 6	1433	,,	12	17	Died of *T. vivax.*
		Average ...	22	56	

ANIMALS SUSCEPTIBLE TO TRYPANOSOMA VIVAX—*continued.*

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.	Remarks.
			Sheep.		
1909.					
Mar. 23	610	Ox 598	—	—	Experiment stopped after 56 days.
Apr. 2	637	Ox 493	—	—	,, ,, 46 ,,
May 19	863	Ox 779	—	—	Died 124 days after inoculation.
June 17	1013	Ox 583	—	—	Experiment stopped after 72 days.
			Monkey.		
Feb. 4	487	Ox 450	—	—	Experiment stopped after 57 days.
,, 4	488	,,	—	—	,, ,, 57 ,,
Apr. 3	640	Ox 583	—	—	,, ,, 45 ,,
,, 3	643	Ox 493	—	—	,, ,, 45 ,,
May 19	859	Ox 779	—	—	,, ,, 40 ,,
July 9	1304	Ox 1030	—	—	Died 30 days after inoculation.
,, 26	1384	Goat 864	—	—	Experiment stopped after 33 days.
			Dog.		
Feb. 4	489	Ox 450	—	—	Died 30 days after inoculation.
Mar. 3	576	,,	—	—	,, 31 ,, ,,
June 18	1021	Ox 583	—	—	,, 28 ,, ,,
,, 30	1197	Goat 864	—	—	Experiment stopped after 43 days.
July 16	1343	Dog 1021	—	—	,, ,, 31 ,,
			Guinea-pig.		
Mar. 27	617	Ox 598	—	—	Experiment stopped after 52 days.
Apr. 3	642	Ox 583	—	—	,, ,, 45 ,,
,, 3	645	Ox 493	—	—	,, ,, 45 ,,
May 19	862	Ox 779	—	—	,, ,, 40 ,,
			Rat.		
Mar. 23	609	Ox 598	—	—	Experiment stopped after 56 days.
Apr. 3	641	Ox 583	—	—	,, ,, 45 ,,
,, 3	644	Ox 493	—	—	,, ,, 45 ,,
May 19	860	Ox 779	—	—	,, ,, 40 ,,
			Mouse.		
Mar. 27	616	Ox 598	—	—	Experiment stopped after 52 ,,
Apr. 3	646	Ox 583	—	—	,, ,, 45 ,,
,, 3	647	Ox 493	—	—	,, ,, 45 ,,
May 19	861	Ox 779	—	—	,, ,, 40 ,,

CULTIVATION OF TRYPANOSOMA VIVAX.

A. *Living, unstained.*

This species grew readily on blood-agar, the blood used in the preparation of the medium being that of the goat. The history of a tube which had been inoculated with a drop of blood from the heart of an ox, Experiment 450, dead of *Trypanosoma vivax* disease, is as follows :—

March 3, 1909.—Inoculated six tubes. The trypanosomes are very scarce in the blood.

March 6.—Tube No. 1 has been examined daily for signs of growth. Nothing living has been seen. The tube remains sterile.

March 8.—To-day many very active trypanosomes are seen. They are single or in small groups of ten or twenty or so. The cell-contents are granular. The cells possess flagella, but no obvious undulating membranes. There are many dividing forms to be seen, and division is evidently going on rapidly, as many individual trypanosomes appear to be undergoing fission into three or more at the same time.

March 9.—Since yesterday an immense multiplication has taken place. The trypanosomes are still very active. They appear to have free flagella and long-pointed, finely granular posterior extremities.

March 11.—Many highly active trypanosomes seen. They are elongated, thin, and have a long free flagellum, sometimes as long as the body itself.

The trypanosomes remained alive and active up to 20 or 30 days, when the tubes became contaminated, or dried up, and were thrown away.

Size.—Cultural forms of *Trypanosoma vivax* vary much in size. At first, when they begin to multiply, on or about the sixth day, the individuals forming groups are about 15 microns in length. Next day they are longer and thinner, and later many are seen 50, 60, or more, microns in length.

Shape.—At first, the young forms are oval or elliptical in shape; afterwards they become more attenuated; and later still may be seen as huge trypanosomes with undulating membranes and long free flagella.

Contents of Cell.—In the living unstained condition this appears to be finely granular.

Undulating Membrane.—In the smaller forms composing the groups no obvious undulating membrane can be made out.

Flagellum.—This is free and well developed.

Motion.—The single forms are extremely active.

B. *Fixed and Stained* (Plate 5).

Protoplasm.—Is homogeneous, but contains many irregular granules and vacuoles.

Nucleus.—Is usually broken up and diffuse.

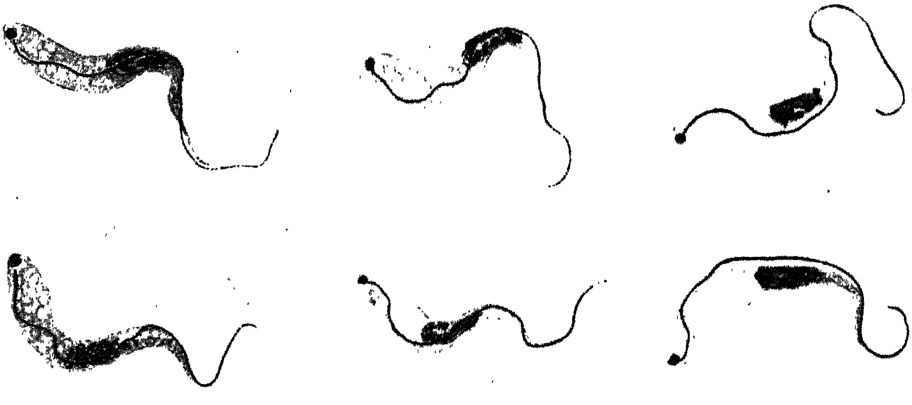
Micronucleus.—Often difficult to distinguish. Sometimes placed anterior, at the side of, or posterior to, the nucleus.

Flagellum.—Well developed free flagellum.

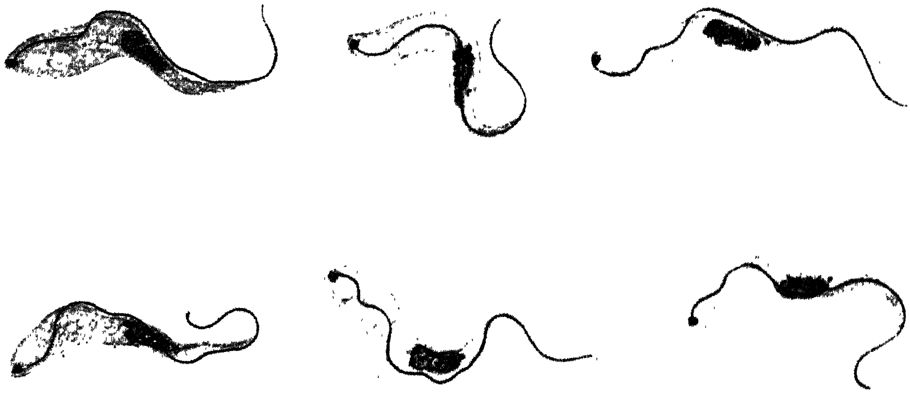
Undulating Membrane.—Present in the older free forms.

Bruce & others

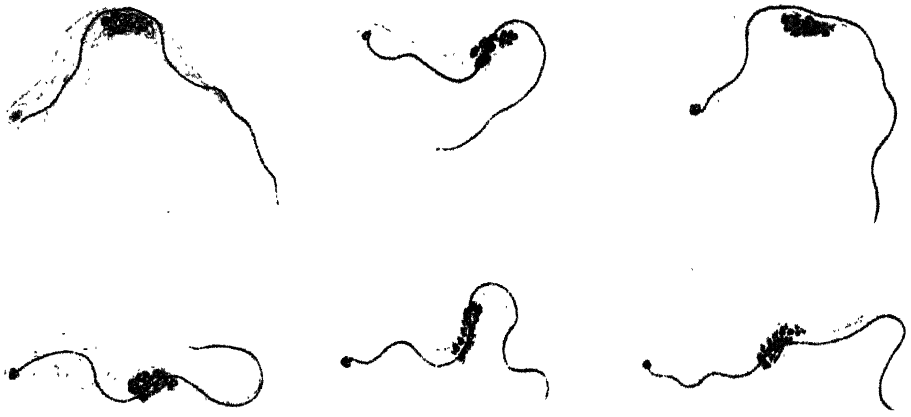
Roy. Soc. Proc. B. vol. 83, Pl. 3.



T. Vivax. Uganda 1909.

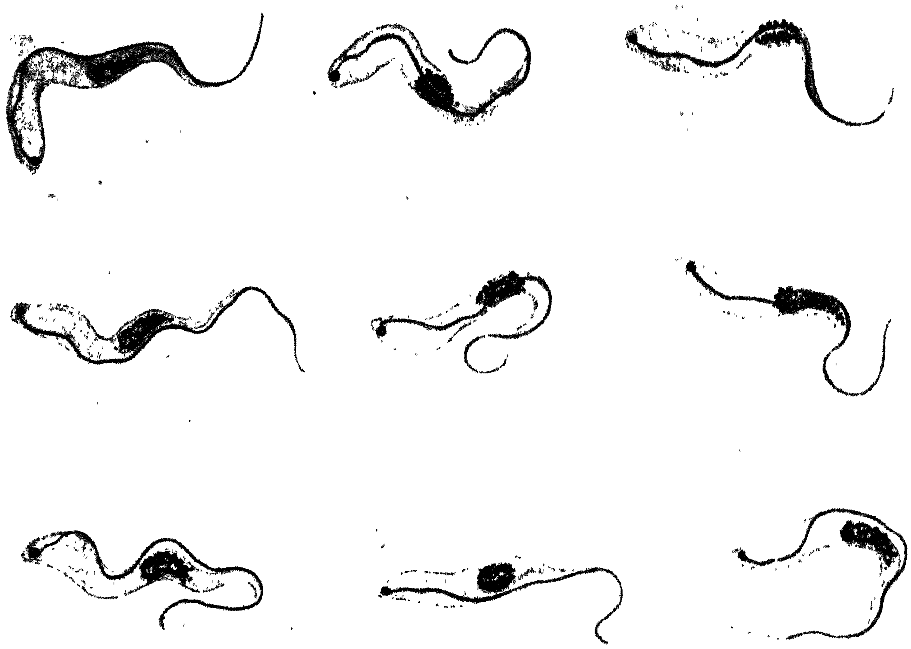


T. Vivax. Pordage's Ox 1903.

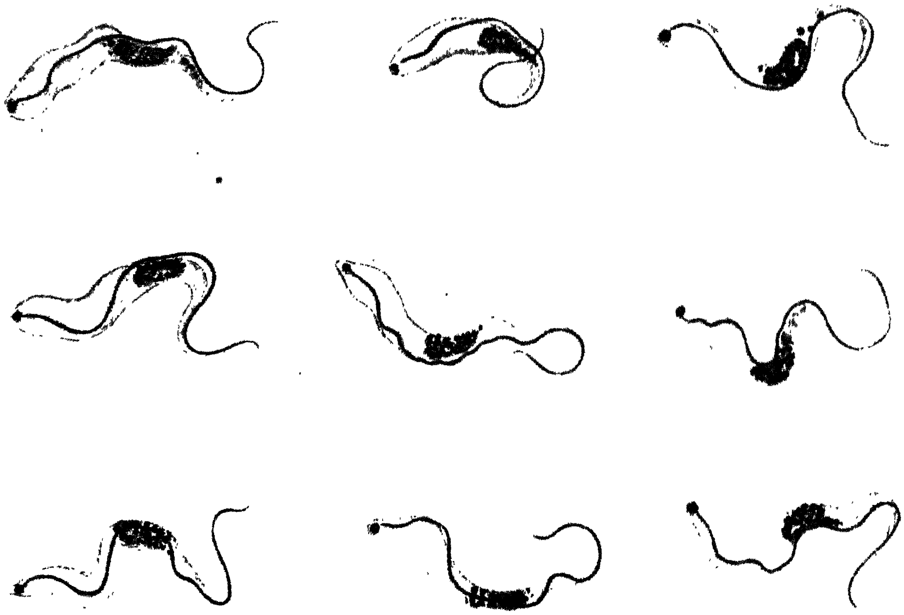


T. Vivax. Ziemann Togoland. 1903.

M. E. Bruce, del.

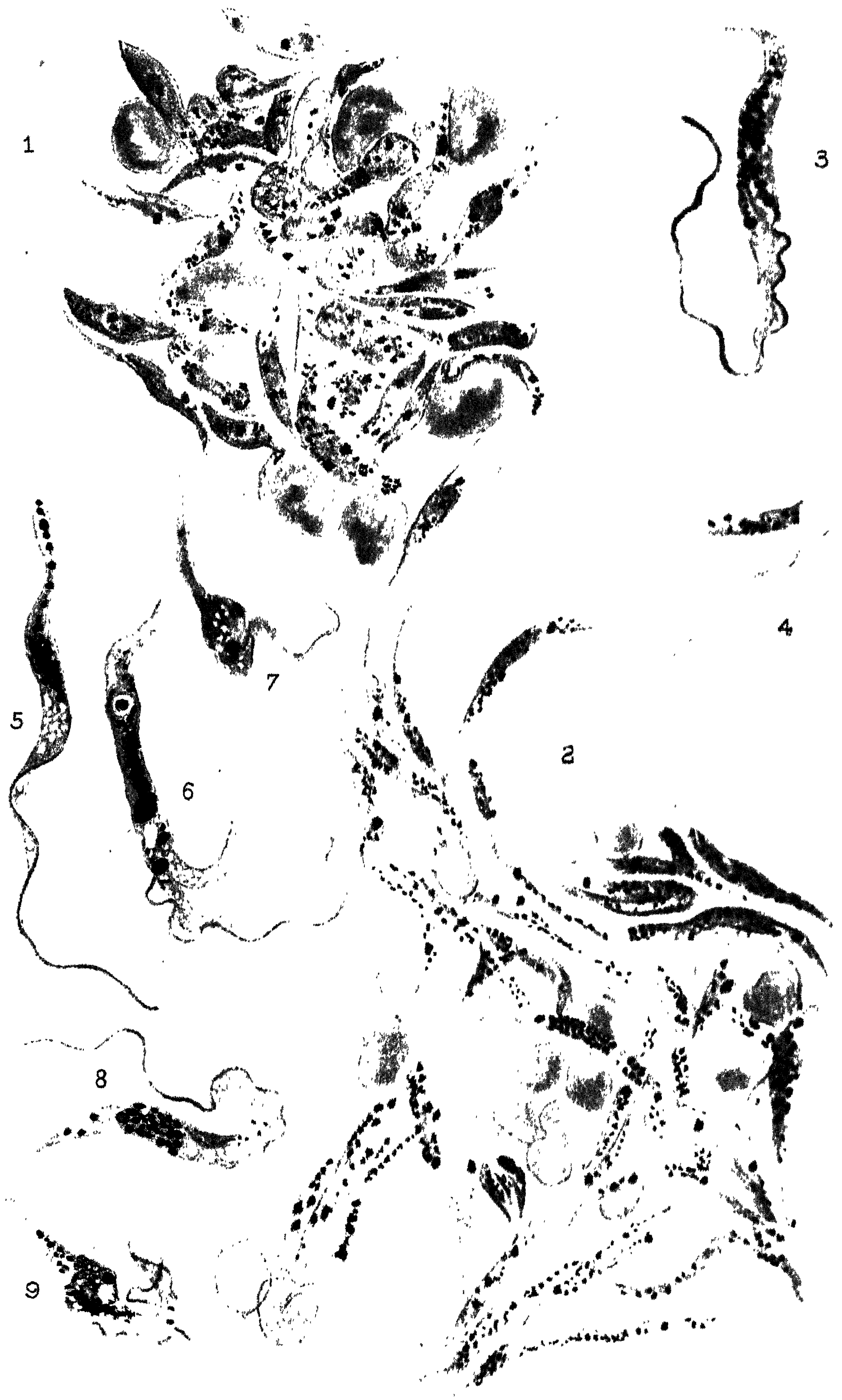


T. Vivax. Ox.



T. Vivax. Goat.

M. E. Bruce, del.



M.E. Bruce, del.

THE CARRIER OF TRYPANOSOMA VIVAX.

It was pointed out in a previous paper that, in laboratory experiments, *Trypanosoma vivax* readily develops in *Glossina palpalis*, and that this tsetse fly can convey the infection to healthy animals after a period of from 20 to 30 days.* It was also found that this fly is naturally infected with this trypanosome, and several animals became ill and died of this disease when freshly-caught Lake-shore flies were fed upon them.† The epidemic among the Government Transport oxen at Kampala also points to this fly being the carrier. As stated above, as long as the cattle were kraaled at the Lake-shore and exposed to the bites of *Glossina palpalis*, so long did they suffer from *Trypanosoma vivax* infection. Afterwards, when stabled at Kampala, the fatality from this disease ceased.

Although it must be confessed the evidence is rather scanty, what there is points to the *Glossina palpalis* as being a carrier, if not the chief carrier, of *Trypanosoma vivax*; and there is no evidence at present to incriminate the *Tabanids* or *Stomoxys*.

CONCLUSIONS.

1. *Trypanosoma vivax*, an easily recognisable species, gives rise to a fatal disease of cattle in Uganda.
2. The carrier of *Trypanosoma vivax* is probably *Glossina palpalis*, which is found naturally infected on the Lake-shore.
3. The reservoir of the virus is possibly the antelope which frequent the *Glossina palpalis* area.

DESCRIPTION OF PLATES.

PLATE 3.

Figures for the comparison of *Trypanosoma vivax*, Uganda, 1909, *Trypanosoma vivax*, Uganda, 1903 (Pordage's ox), and *Trypanosoma vivax*, Togoland, 1903.

PLATE 4.

Trypanosoma vivax, fixed osmic acid, stained Giemsa. ×2000. Note the large round terminal micronucleus; the broad, swollen anterior part filled with clear protoplasm, in which a hint of the alveolar structure is given; the nucleus situated in the narrowed part of the body; the slightly developed undulating membrane; and the free flagellum.

PLATE 5.

Fig. 1.—A group of *Trypanosoma vivax* after 5 days' growth in blood-agar. Stained Giemsa. ×2000.

Fig. 2.—*Trypanosoma vivax* after 6 days' growth. Stained Giemsa. ×2000.

Figs. 3 and 4.—After 7 days' growth. Stained Giemsa. ×2000.

Figs. 5–9.—After 8 days' growth. Stained Giemsa. ×2000.

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* 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 381.

† 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 63.

On the Peculiar Morphology of a Trypanosome from a Case of Sleeping Sickness and the Possibility of its being a New Species (T. rhodesiense).

By J. W. W. Stephens, M.D. Cantab., D.P.H., and H. B. Fantham, D.Sc. Lond., B.A. Cantab.

(Communicated by Prof. Major R. Ross, F.R.S. Received July 19, 1910.)

[Plate 6.]

Prefatory Note.

As already stated in a report to the Advisory Committee for the Tropical Diseases Research Fund, dated May 9, 1910, I noticed early in February, 1910, while examining in class work a stained specimen of rat's blood infected with what was supposed to be *T. gambiense*, a marked peculiarity in the morphology. This peculiarity was so striking that I doubted whether the trypanosome with which I was dealing was really *T. gambiense.* On making enquiries I was told that the strain was derived from a case of Sleeping Sickness then in Prof. Ross's clinic in the Royal Southern Hospital, Liverpool. To make certain that there was no error in this statement I myself infected a rat from the patient's blood. The same forms were, however, again encountered. After convincing myself that these forms were constantly present in infected rats, and that they were not shown by the rats infected with the old laboratory strain of *T. gambiense* maintained at the Runcorn Laboratory, I decided through pressure of work to ask Dr. Fantham (now working in the Liverpool School of Tropical Medicine, under funds allotted by the Advisory Committee for the Tropical Diseases Research Fund) to be so good as to assist me in the description of the morphology of this trypanosome. The following paper is the outcome of our joint work.—[J. W. W. Stephens.]

History of the Strain.

The trypanosomes used during this investigation were obtained from W. A., male, aged 26, a native of Northumberland, who was infected in North-East Rhodesia in September, 1909. It is necessary to set forth the itinerary of W. A. while in Africa, as he was never actually in an area infested with *Glossina palpalis*, so far as records are available, and indeed was never nearer (Kasama) than some 86 miles from such an area.

He first went to South Africa in July, 1904, living in Johannesburg till the end of 1906. He then went to Salisbury for two years. About the end of November, 1908, he left Salisbury for North-Eastern Rhodesia, with a view to prospecting for minerals. The party consisted of two Europeans and ten natives. On the journey northwards he passed through Fort Jameson, Landazi, and Chinsali to Kasama, where he arrived about the beginning of June, 1909. During this northward journey, W. A. passed through an area infested by *Glossina morsitans.* He stayed two months at Kasama, a place from which we have no records of any species of *Glossina.** On the return journey he passed through Mpika (where *Glossina morsitans* occurs), Serenje (no records of *Glossina*), and Mzaza (where there is *Glossina morsitans*). He left Mzaza on September 10, and travelling along the Luangwa River he reached Feira on September 28. During this part of the journey he would pass through an area infested by *Glossina fusca,* between Mzaza and Hargreaves.

He first fell ill on September 20, but after a rest of two days continued his journey. A short stay was made at Feira and then the return journey was continued through the Hartley district to Salisbury, where it was found that he and one native were suffering from trypanosomiasis—parasites being found in his blood in Africa on November 17, 1909.

The patient stated that he had never been near either Lake Tanganyika, Lake Mweru or the Luapula River, which are known to be infested by *Glossina palpalis.* He thought, himself, that he contracted trypanosomiasis while travelling along the Luangwa River, between Mzaza and Feira. The Luangwa Valley is heavily infested with *Glossina morsitans,* but *Glossina palpalis* has not yet been found along the course of this river.

Dr. Bagshawe's article, already noted, on "The Transmission in Nature of *Trypanosoma gambiense,*" should be consulted as to cases of Sleeping Sickness contracted in areas infested by *Glossina morsitans.*

We may note that the case of W. A. has been studied by R. Ross and D. Thomson,† who have found a regular periodical increase in the numbers of the trypanosomes in the peripheral blood of the patient from day to day.

It is also of interest to record that the Rhodesian strain of trypanosome from W. A. is somewhat more virulent to rats and guinea-pigs than the old laboratory strain of *T. gambiense,* a fact already confirmed by other workers in the laboratory. Further, this Rhodesian trypanosome is resistant to atoxyl.

Morphological Features.

It may be stated at once that the peculiarity of this Rhodesian trypanosome is that among the stout or stumpy forms some have the *nucleus at the posterior (non-flagellar) end.*

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* Bagshawe, S.S. Bulletin, No. 18, June, 1910, p. 197.

† 'Roy. Soc. Proc.,' B, 1910, vol. 82, pp. 411–415.

As will be seen from the accompanying figures (Plate 6, figs. 2 to 10), the position of this "posterior" nucleus varies. Starting from the stumpy forms in which the nucleus is in the middle (fig. 1), we have all transitions (figs. 2 to 8) up to that in which the nucleus is actually terminal (fig. 9) and posterior to the blepharoplast (kinetonucleus).

We may meet here any objections that may be raised that the trypanosomes with posterior nucleus are due to distortion, dried films being used, because—

1. We have never found them, though persistently looked for, in films from the same animals infected with the old laboratory strain of *T. gambiense* (figs. 21 to 25), treated in the same way, *i.e.* dried films;

2. Further, we have examined the trypanosomes under discussion, by *intra vitam* staining with methylene blue—by this method the posterior position of the nucleus can be seen; and finally,

3. We have fixed wet films with sublimate-alcohol and with osmic vapour respectively, and subsequently stained them with hæmatoxylin, and found the same forms.

Rats inoculated with the Rhodesian strain usually show a few long thin trypanosomes in the peripheral blood in about three days. The stumpy forms of trypanosomes with the nucleus posterior (figs. 2 to 10) appear about the fifth or sixth day, and from this time onwards somewhat increase in number up to the seventh to eleventh day. They then form about 6 per cent. of the trypanosomes present, but may decrease again, varying from day to day.

These stumpy forms with posterior nucleus (as depicted in figs. 2 to 8) are 17μ to 21μ long and 2μ to 3μ broad. The nucleus often shows a karyosome, and when at the level of the blepharoplast is often kidney-shaped (fig. 8.). There is a well marked blepharoplast (kinetonucleus) and a definite undulating membrane with a flagellar border which terminates in a very short free flagellum. The cytoplasm of these forms is granular, especially at the anterior (flagellar) end, where coarse granules are seen in life, which granules are found to be chromatoid in nature on staining (figs. 2, 3, 5, and 8). These cytoplasmic characters are very like those seen in ordinary stumpy forms of *T. gambiense* (fig. 25). Sometimes a stained line is clearly seen in the stout forms, joining the blepharoplast to the nucleus (fig. 10). We have little evidence as to the relationship of these forms with posterior nucleus to the stumpy forms with the nucleus in the ordinary (central) position (fig. 1), so content ourselves with pointing out that the existence of trypanosomes with posterior nuclei is quite characteristic of this Rhodesian strain, and is so marked a feature that we are always able in films to distinguish it from the old laboratory strain of *T. gambiense*.

These forms with the posterior nuclei are not confined to rats (30 "passages"), but occur also in *Macacus rhesus* (observed by Dr. Yorke), rabbits, and guinea-pigs infected with this strain. We have been unable to detect these forms in the case of Sleeping Sickness itself, for the number of trypanosomes present in the blood was always extremely scanty, and in infected rats showing an equally small number of trypanosomes we have also never been able to find them.

A subsidiary but perhaps not unimportant point in the Rhodesian strain is that many of the ordinary long forms have an elongated posterior end, and may be termed "snout" forms (figs. 13 to 17). The "snout" forms occur more especially during the first half or two-thirds of the period of infection in rats. These elongate forms, while not absent in the old laboratory strain of *T. gambiense*, yet are certainly more numerous in the Rhodesian strain and even by means of this feature we are able to distinguish the strains. "Snout" forms were frequently seen in the blood of W. A. (figs. 14, 16), and in guinea-pigs. Bruce and Nabarro* have already noted these forms with elongate posterior ends.

Again, although forms with the blepharoplast terminal (figs. 18, 19) are found in the Rhodesian strain, they are less numerous than similar trypanosomes with terminal blepharoplast in the old laboratory strain.

We may also note in passing that pear-shaped (fig. 11) or rounded forms with little or no free flagellum are not uncommon in the Rhodesian strain.

Summary and Conclusions.

1. We attach some importance to the fact that the patient (now dead) from whom this strain was derived was never, as far as careful enquiries could elicit, in a *Glossina palpalis* area, but had been in many *Glossina morsitans* areas, and very probably in a small *Glossina fusca* area.

2. These posterior nuclear forms (figs. 2 to 10) have not, as far as we are aware, been described either in the blood of Sleeping Sickness patients or in the blood of animals infected with *T. gambiense*. Seeing how many competent observers have worked with *T. gambiense*, this is a striking point and can hardly be an oversight.

3. We ourselves have been unable to find posterior nuclear forms, though constantly searched for, in the blood of rats infected with the old laboratory strain of *T. gambiense*.

4. Posterior nuclear forms exist also, as is well known, in *T. transvaaliense*, and by Lühe these are regarded as developmental stages of *T. theileri*.

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* Royal Society: Reports of the Sleeping Sickness Commission, No. I, Plate 2, fig. 4.

Whether this be so or not, the condition is not parallel to the one under discussion, for the differences in *T. theileri* are characteristic of all strains of this trypanosome, but the forms which we have described in this paper have, we believe, never been seen before in any strain of *T. gambiense* in man or animals.

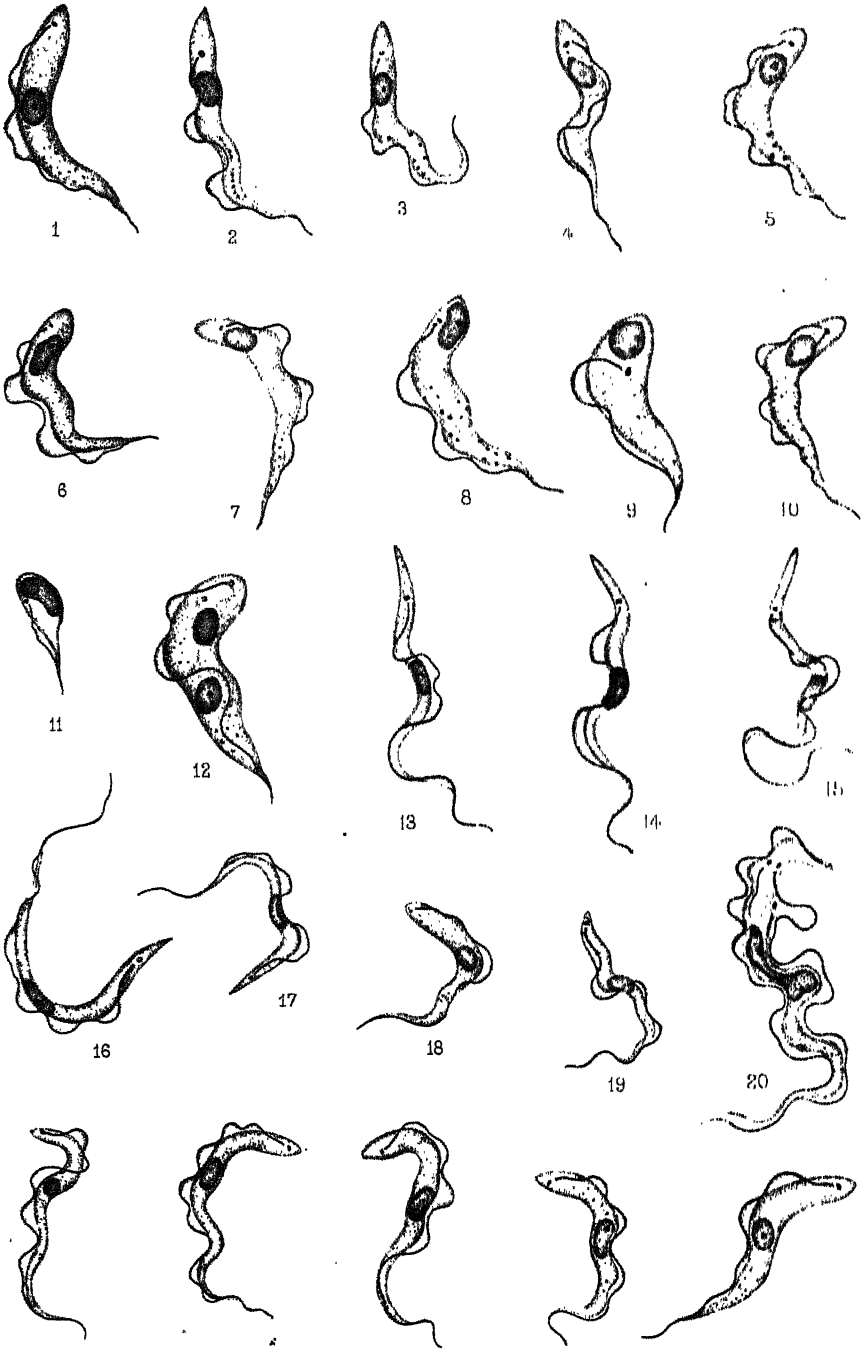
5. We are aware that parasitic flagellates may exhibit morphological variations due to changes in metabolism or to differences in environment. However, this Rhodesian strain of trypanosome with posterior nucleus was seen in rats infected from the patient before he was treated with drugs. At first sight also it might appear that the posterior nucleus denoted merely approaching division or even assumption of the round form. However, we have seen division of these posterior nuclear forms (fig. 12). In any case the peculiar morphological feature exhibited by the Rhodesian trypanosome, in the possession of a posterior nucleus, has not been recorded before.

As to the meaning to be attached to these forms, we will consider the following main possibilities :—

(*a*) That we are dealing with a "variety" or "local race" of *T. gambiense* due to some change of environment. The possibility in this case exists of *T. gambiense* having been conveyed by a species of *Glossina* other than *Glossina palpalis*, which may account for the peculiar morphology we record; but it must be added that we have no knowledge of any other trypanosome undergoing similar changes owing to such a cause. That it is a "laboratory" variation we think is out of the question, as all the known laboratory strains of *T. gambiense* are practically identical in morphology.

(*b*) That we are dealing with a new species of trypanosome also producing Sleeping Sickness in man. In support of this view there is firstly, the morphology, which considered alone is strong evidence, since the Rhodesian trypanosome differs from *T. gambiense* more than, for instance, *T. brucei* does from *T. evansi*. Secondly, there is the history, taken in connection with the fact that, so far as we know, *T. gambiense* is conveyed solely by *Glossina palpalis*, but even should it prove that *Glossina palpalis* exists in the districts through which W. A. travelled, then all the more probable is it that we are dealing with a new species of trypanosome.

It seems to us, however, that it is difficult in the absence of further knowledge to discriminate with certainty between these views. Yet, on account of its peculiar morphological features, this Rhodesian trypanosome at least merits a distinct designation as *T. gambiense rhodesiense*, adopting the trinomial nomenclature. Our own view, however, is that we are dealing with a new species of human trypanosome for which we propose the name *Trypanosoma rhodesiense*.



H. B. Fantham del.

Wood, Newman lith.

EXPLANATION OF PLATE No. 6.

All figures drawn with Abbé camera lucida, using 2 mm. apochromatic objective and 12 compensating ocular (Zeiss). Magnification 1800 diameters approximately.

Figs. 1–20.—Rhodesian strain of human trypanosome. Figures drawn from parasites in the blood of rats, except where otherwise stated.

Fig. 1.—Stout form with nucleus median.
Figs. 2–8.—Stout and stumpy forms, each with posterior nucleus. The nucleus is seen gradually to become more posterior, till it lies behind the blepharoplast (fig. 9).
Fig. 10 —Posterior nuclear form with line connecting blepharoplast and nucleus.
Fig. 11.—Posterior nuclear form becoming rounded.
Fig. 12.—Posterior nuclear form showing division.
Figs. 13–17.—"Snout" forms. (Figs. 13, 15 from rat's blood, figs. 14, 16, 17 from man.)
Figs. 18, 19.—Forms with terminal blepharoplast.
Fig. 20.—Multiple division form, with four blepharoplasts and two nuclei. Such parasites are not uncommon in the Rhodesian strain.

Figs. 21–25.—Various trypanosomes drawn from the blood of rats infected with the old laboratory strain of *T. gambiense.*

The Influence of Bacterial Endotoxins on Phagocytosis, including a New Method for the Differentiation of Bacteria.

(Second Report.)

By Leonard S. Dudgeon, P. N. Panton and H. A. F. Wilson.

(Communicated by Dr. F. W. Mott, F.R.S. Received August 2,—Read November 17, 1910.)

(From the Pathological Laboratories, St. Thomas's Hospital.)

It was shown in a paper communicated by us to the Royal Society in April, 1910—

(*a*) That bacterial endotoxins have the power of inhibiting phagocytosis; that in some cases this action is general, but in most cases it is specific.

(*b*) That the endotoxic substance is unaltered by prolonged exposure to high temperatures.

(*c*) That, as far as our experiments then carried us, the inhibition of phagocytosis appeared to result from an interaction between endotoxin and serum.

The further investigations which form the basis of this communication have been mainly directed towards the elucidation of the mode of action of the endotoxic substance. Firstly, whether it acts on the serum, the leucocytes, or the bacteria; secondly, how far this action is specific for various organisms belonging to the same family; and thirdly, whether the inhibition of phagocytosis is due to absorption of complement by the endotoxin.

In the present series of experiments the technique adopted has been the same as described in the preliminary report, with the exception that the bacteria were allowed to grow for a longer period of time, so as to obtain a more abundant growth for the preparation of the endotoxin. In every case the bacterial deposit was alternately frozen by means of solid carbon dioxide and thawed, as by this method a more potent extract was obtained. For each group of experiments a fresh extract was prepared.

Is the action of the Endotoxic Substance on the Leucocytes?

Equal parts of the extract and washed human leucocytes were mixed together and incubated for one hour at 37° C.; they were then centrifugalised and the supernatant fluid pipetted off. One volume of the treated leucocytes was added to equal volumes of serum and bacillary suspension and incubated for 15 minutes at 37° C. In every instance the leucocytes were found to be unaffected, since as marked phagocytosis occurred when the leucocytes had been submitted to this treatment as in the control. The following brief experiment will serve to illustrate this point:—

	No. of bacilli in 50 cells.	No. of non-phagocytic cells.
A. (Normal leucocytes + saline) + normal serum + *B. Achard*	328	1
" " + " + *B. typhosus*	132	4
B. (Normal leucocytes + Achard extract) + normal serum + *B. Achard*	300	2
" " " + " + *B. typhosus*	138	4
C. (Normal leucocytes + typhoid extract) + normal serum + *B. Achard*	369	0
" " " + " + *B. typhosus*	156	8

Is the action of the Endotoxic Substance on the Bacteria themselves?

For this series of experiments a given volume of extract was added to an equal volume of bacteria and incubated for one hour at 37° C. The mixture was then centrifugalised at very high speed until a clear supernatant fluid was obtained; this was pipetted off and an equal volume of normal saline was added to the deposit and the whole thoroughly mixed. A volume of this final bacterial mixture was then added to equal volumes of normal serum and leucocytes. The result of these experiments was to show that no action took place between the organisms and the endotoxic substance, and that phagocytosis was unaffected. It is only necessary to cite one experiment, as all our results were identical:—

	No. of bacilli in 50 cells.	No. of non-phagocytic cells.
A. Normal saline + *B. coli* + normal leucocytes + normal serum......	361	0
„ + *B. Danysz* + normal leucocytes + normal serum	313	1
B. Coli extract + *B. coli* + normal leucocytes + normal serum	420	0
„ + *B. Danysz* + normal leucocytes + normal serum ...	339	0
C. Danysz extract + *B. coli* + „ + „ ...	449	0
„ + *B. Danysz* + normal leucocytes + normal serum	385	0

Is the action of the Endotoxic Substance on the Serum?

As it was evident that the action of this body was not directly related to the leucocytes or the organisms themselves, further investigations, mostly of a confirmatory nature, were undertaken in connection with its action on the serum. The same technique has been employed as given in detail in our previous report.

Numerous experiments were made for the purpose of ascertaining whether absorption of complement and inhibition of phagocytosis, resulting from the action of endotoxin upon normal serum, were directly related. It was found, however, that no greater phagocytosis occurred in those instances where ample hæmolytic complement was still present at the end of the period of interaction than if all the complement had been absorbed by the endotoxic substance. For the hæmolytic experiments either sheep's red cells or sensitised human red cells were employed. The following experiment may be cited to illustrate these points:—

	No. of bacilli in 50 cells.	No. of non-phagocytic cells.	Hæmolytic experiments.
A. Serum saline* + leucocytes + *B. typhosus*	100	8	Complete hæmolysis.
„ „ + „ + *B. paratyphoid*	118	3	
B. Normal serum paratyphoid extract + leucocytes + *B. typhosus* ...	65	16	No hæmolysis.
„ „ „ + leucocytes + *B. paratyphoid*	16	40	
C. Normal serum typhoid extract + leucocytes + *B. typhosus*	8	43	No hæmolysis.
„ „ „ + „ + *B. paratyphoid*	90	7	

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* By "serum saline," "serum paratyphoid extract," etc., is meant one volume of a mixture of equal parts of serum and saline, or of serum and the bacterial extract in question, after one hour's incubation at 37° C.

Experiment C of this series serves to show that, while the hæmolytic complement was completely absorbed by the endotoxin, the degree of phagocytosis of the foreign organism was little altered.

Specificity.

In the experiment referred to below, the specific action of the endotoxin is clearly shown. We have already explained at the commencement of this paper that when the endotoxin and leucocytes are incubated together there is no reduction in the amount of phagocytic activity as occurs here.

	No. of bacilli in 50 cells.	No. of non-phagocytic cells.
A. Serum saline + leucocytes + *B. Achard*	328	1
„ + „ + *B. typhosus*	132	4
B. Serum Achard extract + leucocytes + *B. Achard*..............	40	26
„ „ + „ + *B. typhosus*	176	5
C. Serum typhoid extract + leucocytes + *B. Achard*	155	6
„ „ „ + „ + *B. typhosus*	0	50

The specific nature of this reaction, even between such closely allied organisms as *B. typhosus* and *B. Achard*, is thus proved beyond question. Similar results were obtained with many other members of the typhoid-coli-paratyphoid family. Further, experiments were made to determine whether organisms of the same species, but derived from different sources, would yield an endotoxin with identical properties or not.

In the following instance two strains of *B. typhosus* were used. One strain was isolated from a gall-bladder and has been frequently sub-cultured for the last four years, while the other was recently isolated from the blood of a patient suffering from typhoid fever:—

	No. of bacilli in 50 cells.	No. of non-phagocytic cells.
A. Serum saline + leucocytes + *B. typhosus* (bile)	219	1
„ + „ + *B. typhosus*	126	5
B. Serum typhoid extract + leucocytes + *B. typhosus* (bile)	3	48
„ „ + „ + *B. typhosus*	7	46
C. Serum typhoid (bile) extract + leucocytes + *B. typhosus* (bile) ...	5	45
„ „ + „ + *B. typhosus*...........	3	48

It would thus appear that this method can be employed for the purpose of differentiating bacteria. Bacteria so closely related as the typhoid-paratyphoid roup can be separated, so that there is every prospect that this method may prove of considerable value in bacteriological investigations. Further experiments are now being made with other organisms which up to the present time have not been proved to be absolutely distinct.

Numerous experiments were made on the resistance of these endotoxins to heat, and every result shows that they are thermostable even when exposed to a temperature of 100° C. for 30 minutes, and are still able to exert their specific action, as is shown by the following experiment:—

	No of bacilli in 50 cells.	No. of non-phagocytic cells.
A. Serum saline + leucocytes + *B. coli*	233	0
B. Serum Danysz extract + leucocytes + *B. coli*	59	15
C. Serum coli extract + leucocytes + *B. coli*	4	46
D. Serum Danysz extract* + leucocytes + *B. coli*	59	16
E. Serum coli extract* + leucocytes + *B. coli*	6	44

* This extract was exposed to a temperature of 100° C. for 30 minutes.

On the Determination of the Chief Correlations between Collaterals in the Case of a Simple Mendelian Population Mating at Random.

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(Communicated by Prof. Karl Pearson, F.R.S. Received June 3,—Read June 30, 1910.)

(1) In a paper communicated to the Royal Society in April, 1909, Prof. Pearson obtained the gametic correlations between the offspring and the ancestry in each grade in a simple Mendelian population mating at random. By a "simple Mendelian population" for a given character, he understood one which started with any definite ratio of dominant individuals (AA) to recedents (*aa*). These mating at random give rise to a population which may be written in the form

$$p^2\,(\mathrm{AA}) + 2pq\,(\mathrm{A}a) + q^2\,(aa),$$

and of which, without selection, the proportions of dominants, recedents, and hybrids are known to remain constant, with continued random mating, during successive generations. Prof. Pearson found that, both in the case of gametic and somatic characters, the ancestral correlations diminished in a geometrical progression, and thus obeyed the fundamental principle of the Law of Ancestral Heredity, as deduced from observations on man and other living forms.

(2) It is difficult to believe that the characters dealt with in the case of Mendelian investigations on animals can be largely affected by environment, but it is easy to allow for this influence by the method of partial correlation. If, in an investigation on any given character, the subscript 1 denote offspring, 2 the ancestor in any generation, 3 the offspring's environment, and 4 the ancestor's environment, then the correlation between 1 and 2 for constant 3 and 4 is given by

$${}_{34}\rho_{12} = \frac{r_{12}(1-r_{34}^2)-r_{13}r_{23}-r_{14}r_{24}+r_{34}(r_{14}r_{23}+r_{13}r_{24})}{\{1-r_{23}^2-r_{34}^2-r_{24}^2+2r_{23}r_{34}r_{24}\}^{\frac{1}{2}}\{1-r_{13}^2-r_{14}^2-r_{34}^2+2r_{13}r_{34}r_{14}\}^{\frac{1}{2}}}.$$

If we suppose the environment of a given stock to remain constant, this reduces to the simple form

$${}_{e}\rho_{12} = \frac{r_{12}-e^2}{1-e^2},$$

where e is the correlation between character and environment.* This should be absolutely true for pairs of brothers reared in the same home conditions.

Many investigations into the value of e for a great variety of characters in man have been carried out in the Statistical Laboratory at University College, and these show that the value of e is remarkably small. Its average value is 0·03, and it rarely reaches an intensity as high as 0·1. It can only introduce a second order correction into the correlation, and one well within the limits of the probable error of the determination. If e were 0·1, the change in fraternal correlation would only be from 0·50 to 0·495. Thus, until the number and accuracy of observations have reached a much higher stage, the influence of environment need not be considered.

(3) A noteworthy conclusion reached by biometric workers by actual measurements on man is that the correlation of brothers is not much larger than the correlation of parent and offspring. *A priori*, we

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* A German writer has recently given a formula for correcting fraternal correlation for the influence of environment. It differs from the above form, and consists in a factor multiplying r_{12}. He assumes that the high values found for the parental and fraternal correlations in man as compared with those to be expected for *somatic* characters in the Mendelian hypothesis are due to the neglect of the influence of environment by the biometricians. He was probably unaware of the amount of attention which has been given by the biometric school to the determination of environmental correlations and to the comparison of the correlations for characters where environment is likely and unlikely to produce effect, *e.g.* heredity in stature and forearm with heredity in eye-colour and cephalic index. See W. Weinsberg, 'Zeitschrift für Vererbungslehre' 1909, vol. 1.

should expect it to be larger, for brothers have two parents in common, while between father and son comes in the influence of the mother, exerted only on the latter member of the pair. Thus Sir Francis Galton, in his 'Natural Inheritance' (p. 133), makes the fraternal resemblance *twice* as great as the paternal. Pearson*, dealing with three characters in 12 tables of over 1000 each, found the average correlation for parent and child to be 0·46, and for three characters in nine tables the average fraternal correlation to be 0·52. For eye-colour in man† he found the parental correlation 0·495 and the fraternal 0·475, showing a slightly reversed relationship. Both, however, compel us to affirm that the degrees of resemblance between parent and offspring and between brethren are very nearly equal and differ but slightly from 0·5. The values which Mendelian theory leads us to expect are shown in Table IX.

(4) Further conclusions can be drawn now that the measurement of resemblance between pairs of cousins and between uncles or aunts and nephews or nieces has been completed. Judged by a great variety of characters, the cousin relationship has been found to be as close as, if not closer than, the avuncular relationship. This result is, of course, no more remarkable than that between the parental and fraternal resemblances referred to above. However, in view of the publication of a large number of avuncular correlations which have been worked out in the Statistical Laboratory at University College, it seems desirable to show that it is at least consistent with some one or other theory of heredity. From Table IX it will be seen that on the Mendelian theory the relationship between cousins is as close as that between uncle and nephew.

The point is one which is of considerable importance in medical diagnosis. The parents' brothers and sisters have been usually included, and the cousins excluded, in considering the family history. The fact, however, that a man's cousins resemble him as closely as do his uncles and aunts shows that the cousins, usually more numerous, provide at least as good material for an estimate of his tendencies as his parents' brothers and sisters.

The paradox that a man's father's brother's son resembles him, on the average, as closely as his father's brother, although the additional influence of the father's brother's wife has come in, is only a paradox so long as we overlook the fact that somatic and gametic characters are not causally related but only correlated. Almost any determinantal theory of heredity brings out the fact that, on the average, a man resembles his father as closely as he does his brother, and, further, that he is not more like to his uncle than to his uncle's son. This point is illustrated in the present paper from the investigation of the collateral correlations of a simple Mendelian population mating at random because that determinantal theory is much in vogue to-day. It will be used here to indicate that the facts reached by observation as to the degrees of resemblance between an individual and his ascendants and collaterals, and considered by some to be paradoxical, are curiously enough legitimate developments of Mendelian theory. If they are paradoxes of biometric observations, they are also paradoxes of Mendelian theory, and highly probably of other determinantal hypotheses which may hereafter be developed.

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* 'Biometrika,' vol. 2, pp. 378, 387.

† 'Phil. Trans.,' A, 1900, vol. 195, p. 106.

(5) The three first degrees of collateral relationship are worked out in the following paragraphs, viz.:—

(A) The correlation of siblings, *i.e.* of brethren regardless of sex.
(B) The correlation of uncles and aunts with nephews and nieces.*
(C) The correlation of cousins regardless of sex.

The algebra presents no special difficulties beyond laboriousness.

(6) Starting with the population of the form

$$p^2(\mathrm{AA})+2pq(\mathrm{A}a)+q^2(aa),$$

after $16s$† random matings of pairs of individuals of each class, the types of families produced will be those given in the square brackets in Table I. The frequencies of the families will be the factors which multiply the expressions within the brackets. Thus, for example, the mating of the p^2 individuals possessing the protogenic constituent (AA) with the $2pq$ possessing the hybrid (Aa) gives rise to $2p^3q$ families, in each of which $8s$ individuals have the protogenic constituent (AA) and $8s$ the hybrid constituent (Aa).

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* The word "siblings" has been reintroduced and largely accepted as a convenient term for brethren of both sexes, but no like terms are in use to denote uncle or aunt and nephew or niece regardless of sex. Prof. Pearson has suggested *eldersib* for uncle or aunt and *sibmag* for nephew or niece as convenient Anglo-Saxon names.

† The number of matings is taken of the form $16s$ in order to prevent fractions occurring later on in the paper. See Table IV.

Table I.

Second parent.	First parent.		
	p^2 (AA).	$2pq$ (Aa).	q^2 (aa).
p^2 (AA)	$p^4\,[16s\,(\mathrm{AA})]$	$2p^3q\,[8s\,(\mathrm{AA})+8s\,(\mathrm{A}a)]$	$p^2q^2\,[16s\,(\mathrm{A}a)]$
$2pq$ (Aa)	$2p^3q\,[8s\,(\mathrm{AA})+8s\,(\mathrm{A}a)]$	$4p^2q^2\,[4s\,(\mathrm{AA})+8s\,(\mathrm{A}a)+4s\,(aa)]$	$2pq^3\,[8s\,(\mathrm{A}a)+8s\,(aa)]$
q^2 (aa)	$p^2q^2\,[16s\,(\mathrm{A}a)]$	$2pq^3\,[8s\,(\mathrm{A}a)+8s\,(aa)]$	$q^4\,[16s\,(aa)]$

(A.) *Correlation between Brothers.*

(7) From Table I we can pick out all the pairs of brothers and arrange them according to the gametic character exhibited by each member of the pair. The cases which can arise, and the number of pairs of brothers in each case are:—

Both brothers (AA)—

$$p^4 . \tfrac{1}{2} . 16s(16s-1)+2p^3q . \tfrac{1}{2} . 8s(8s-1)$$
$$+2p^3q . \tfrac{1}{2} . 8s(8s-1)+4p^2q^2 . \tfrac{1}{2} . 4s(4s-1).$$

Both brothers (Aa)—

$$2p^3q . \tfrac{1}{2} . 8s(8s-1)+p^2q^2 . \tfrac{1}{2} . 16s(16s-1)+2p^3q . \tfrac{1}{2} . 8s(8s-1)$$
$$+4p^2q^2 . \tfrac{1}{2} . 8s(8s-1)+2pq^3 . \tfrac{1}{2} . 8s(8s-1)+p^2q^2 . \tfrac{1}{2} . 16s(16s-1)$$
$$+2pq^3 . \tfrac{1}{2} . 8s(8s-1).$$

Both brothers (aa)—

$$4p^2q^2 . \tfrac{1}{2} . 4s(4s-1)+2pq^3 . \tfrac{1}{2} . 8s(8s-1)+2pq^3 . \tfrac{1}{2} . 8s(8s-1)$$
$$+q^4 . \tfrac{1}{2} . 16s(16s-1).$$

One brother (AA), other brother (Aa)—

$$2p^3q . 8s . 8s+2p^3q . 8s . 8s+4p^2q^2 . 4s . 8s.$$

One brother (AA), other brother (aa)—

$$4p^2q^2 . 4s . 4s.$$

One brother (Aa), other brother (aa)—

$$4p^2q^2 . 8s . 4s+2pq^3 . 8s . 8s+2pq^3 . 8s . 8s.$$

From the above expressions a symmetrical table can be constructed, from which to obtain the *gametic* correlation between brothers. This is given in Table II. By arranging the above expressions under the headings "A" and "not A" another table can be formed to give the *somatic* correlation. This is given in Table III. In both tables the factor $16s$, which multiplies every term occurring, has, for brevity, been omitted.

Table II.—Gametic Correlation between Brothers.
(Factor $16s$ omitted throughout.)

Second brother.	First Brother.			Totals.
	(AA.)	(A*a*.)	(*aa*.)	
(AA)	$(16s-1)p^4+(16s-2)p^3q$ $+(4s-1)p^2q^2$	$16sp^3q+8sp^2q^2$	$4sp^2q^2$	$(16s-1)p^2(p+q)^2$
(A*a*)	$16sp^3q+8sp^2q^2$	$(16s-2)p^3q+4(12s-1)p^2q^2$ $+(16s-2)pq^3$	$8sp^2q^2+16spq^3$	$2(16s-1)pq(p+q)^2$
(*aa*)	$4sp^2q^2$	$8sp^2q^2+16spq^3$	$(4s-1)p^2q^2+(16s-2)pq^3$ $+(16s-1)q^4$	$(16s-1)q^2(p+q)^2$
Totals	$(16s-1)p^2(p+q)^2$	$2(16s-1)pq(p+q)^2$	$(16s-1)q^2(p+q)^2$	$(16s-1)(p+q)^4$

Table III.—Somatic Correlation between Brothers.
(Factor $16s$ omitted throughout.)

Second brother.	First brother.		Totals.
	(A.)	(Not A.)	
(A)	$(16s-1)p^4+4(16s-1)p^3q+(68s-5)p^2q^2$ $+(16s-2)pq^3$.	$12sp^2q^2+16spq^3$	$(16s-1)p(p+q)^2(p+2q)$
(Not A)	$12sp^2q^2+16spq^3$	$(4s-1)p^2q^2+(16s-2)pq^3$ $+(16s-1)q^4$	$(16s-1)q^2(p+q)^2$
Totals	$(16s-1)p(p+q)^2(p+2q)$	$(16s-1)q^2(p+q)^2$	$(16s-1)(p+q)^4$

Since the standard deviations of the totals in these tables are the same in both directions, the correlations can at once be found from a knowledge of the means of the columns.

(8) *Gametic Correlation.*—If, in Table II, $\bar{y}_1$, $\bar{y}_2$, $\bar{y}_3$, and $\bar{y}$ are the means of the first, second, and third columns, and of the whole table respectively, each being measured from the centre of the middle row, we find

$$\bar{y}_1=\frac{(16s-1)p^2+(16s-2)pq-q^2}{(16s-1)(p+q)^2}=\frac{(16s-1)p-q}{(16s-1)(p+q)},$$

$$\bar{y}_2=\frac{8s(p^2-q^2)}{(16s-1)(p+q)^2}=\frac{8s(p-q)}{(16s-1)(p+q)},$$

$$\bar{y}_3=\frac{p^2-(16s-2)pq-(16s-1)q^2}{(16s-1)(p+q)^2}=\frac{p-(16s-1)q}{(16s-1)(p+q)},$$

$$\bar{y}=\frac{16s(16s-1)(p+q)^2(p^2-q^2)}{16s(16s-1)(p+q)^4}=\frac{p-q}{p+q}.$$

Hence $$\bar{y}_1-\bar{y}_2=\frac{8s-1}{16s-1}=\bar{y}_2-\bar{y}_3=r,$$

from which it follows that the regression is linear and the correlation is $\frac{8s-1}{16s-1}$.

(9) *Somatic Correlation.*—In this case, from Table III, we have, measuring from the "not A" row,

$$\bar{y}_1=\frac{(16s-1)p^3+4(16s-1)p^2q+(68s-5)pq^2+(16s-2)q^3}{(16s-1)(p+q)^2(p+2q)},$$

$$\bar{y}_2=\frac{12sp^2+16spq}{(16s-1)(p+q)^2},$$

$$\bar{y}=\frac{p(p+2q)}{(p+q)^2},$$

$$\bar{y}_1-\bar{y}_2=\frac{(4s-1)p+(16s-2)q}{(16s-1)(p+2q)}=r.$$

The *somatic* correlation therefore depends upon the ratio of p to q, *i.e.* upon the ratio of the number of individuals in the population possessing the dominant character to the number possessing the recedent. For different values of p and q the above value of r can range between $\frac{4s-1}{16s-1}$ and $\frac{1}{2}\cdot\frac{16s-2}{16s-1}$. If $p=q$ its value is $\frac{20s-3}{3(16s-1)}$.*

(10) In considering the value to be given to s in these formulæ in order that they can be compared with statistical results, we must remember that the latter are obtained from families of varying size, from which we take selections of fraternal couples. We must therefore look upon our observations as forming a random sample of all the possible pairs of brethren obtained from an indefinitely large number of matings. That is, we must make s indefinitely large. Then we get the *gametic* correlation to be $\frac{1}{2}$, and the range for the *somatic* correlation from $\frac{1}{4}$ to $\frac{1}{2}$, with the value $\frac{5}{12}$ when $p=q$.

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* This particular value of the *somatic* fraternal correlation was reached by Prof. Pearson in his memoir in the 'Phil. Trans.,' A, 1904, vol. 203. In that memoir he dealt with *somatic* characters only, and he assumed the population to be generated from a series of hybridisations and not from a combination of individuals exhibiting the protogenic, allogenic and hybrid constituents in certain proportions. But he was more general in his supposition that the character depended upon n Mendelian couplets, and not on a single one.

(B.) *Correlation between Uncle and Nephew.*

(11) In this case we require not only the various types of families given in Table I, but also the offspring of these families when mated at random. But it will not be sufficient to mate the nine types of families given by Table I with the general population in which the proportions possessing the protogenic, hybrid, and allogenic constituents are p^2(AA), $2pq$(Aa), and q^2(aa), for any particular mating will be of a member of one type of family with a member of the same or another type. An avuncular relationship will arise with the members of both families from which the mating couples are selected. Thus we must discover the offspring which arise from the mating of each type of family in Table I with itself and with each other. This is given in Table IV, in which $4t$ is taken to be the number of matings of each pair. This allows for a possible change of fertility in consecutive generations.

(12) To explain Table IV, consider, for example, the mating of the family of type $4s$(AA)$+8s$(Aa)$+4s$(aa) [Type α] with the family of type $8s$(AA)$+8s$(Aa) [Type β]. Of the $8s$(AA) of Type β:—

- $2s$ will mate with $2s$ of the $4s$(AA) from Type α, giving $4t\{2s(\mathrm{AA})\}$ offspring.
- $4s$ will mate with $4s$ of the $8s$(Aa) from Type α, giving $2t\{4s(\mathrm{AA})\}+2t\{4s(\mathrm{A}a)\}$ offspring.
- $2s$ will mate with $2s$ of the $4s$(aa) from Type α, giving $4t\{2s(\mathrm{A}a)\}$ offspring.

Of the $8s$(Aa) of Type β:—

- $2s$ will mate with $2s$ of the $4s$(AA) from Type α, giving $2t\{2s(\mathrm{AA})\}+2t\{2s(\mathrm{A}a)\}$ offspring.
- $4s$ will mate with $4s$ of the $8s$(Aa) from Type α, giving $t\{4s(\mathrm{AA})\}+2t\{4s(\mathrm{A}a)\}+t\{4s(aa)\}$ offspring.
- $2s$ will mate with $2s$ of the $4s$(aa) from Type α, giving $2t\{2s(\mathrm{A}a)\}+2t\{2s(aa)\}$ offspring.

The complete result will consist of the aggregation of the six combinations written out. Since the frequency of the Type α family is $4p^2q^2$, and of the Type β is $4p^3q$, there will be altogether $16p^5q^3$ such aggregations as the above. The result is represented in the table, in which the families derived through any particular individual exhibiting the constituent (AA), (Aa), or (aa) are written in the same horizontal or vertical line as the constituent itself. The remaining aggregations can be read off in like manner.

(13) From Table IV we can pick out uncle and nephew in pairs according

Table IV.—Showing the Types and Frequencies of the Families of the Second Generation.

	$\overbrace{16s(AA)}^{p^4}$	$\overbrace{8s(AA) + 8s(Aa)}^{4p^3q}$	$\overbrace{4s(AA) + 8s(Aa) + 4s(aa)}^{4p^2q^2}$	$\overbrace{16s(Aa)}^{2p^2q^2}$	$\overbrace{8s(Aa) + 8s(aa)}^{4pq^3}$	$\overbrace{16s(aa)}^{q^4}$
$p^4\{16s(AA)$	p^8 $4t[16s(AA)]$	$4p^7q$ $4t[8s(AA)]+\begin{array}{l}2t[8s(AA)]\\+2t[8s(Aa)]\end{array}$	$4p^6q^2$ $4t[4s(AA)]+\begin{array}{l}2t[8s(AA)]\\+2t[8s(Aa)]\end{array}+4t[4s(Aa)]$	$2p^6q^2$ $\begin{array}{l}2t[16s(AA)]\\+2t[16s(Aa)]\end{array}$	$4p^5q^3$ $\begin{array}{l}2t[8s(AA)]\\+2t[8s(Aa)]\end{array}+4t[8s(Aa)]$	p^4q^4 $4t[16s(Aa)]$
$4p^3q\left\{\begin{array}{l}8s(AA)\\+\\8s(Aa)\end{array}\right.$	$4p^7q$ $4t[8s(AA)]$ $+$ $\begin{array}{l}2t[8s(AA)]\\+2t[8s(Aa)]\end{array}$	$16p^6q^2$ $4t[4s(AA)]+\begin{array}{l}2t[4s(AA)]\\+2t[4s(Aa)]\end{array}$ $+\qquad+$ $\begin{array}{l}2t[4s(AA)]\\+2t[4s(Aa)]\end{array}\quad\begin{array}{l}t[4s(AA)]\\+2t[4s(Aa)]\\+t[4s(aa)]\end{array}$	$16p^5q^3$ $4t[2s(AA)]+\begin{array}{l}2t[4s(AA)]\\+2t[4s(Aa)]\end{array}+4t[2s(Aa)]$ $+\qquad+\qquad+$ $\begin{array}{l}2t[2s(AA)]\\+2t[2s(Aa)]\end{array}\quad\begin{array}{l}t[4s(AA)]\\+2t[4s(Aa)]\\+t[4s(aa)]\end{array}\quad\begin{array}{l}2t[2s(Aa)]\\+2t[2s(aa)]\end{array}$	$8p^5q^3$ $\begin{array}{l}2t[8s(AA)]\\+2t[8s(Aa)]\end{array}$ $+$ $\begin{array}{l}t[8s(AA)]\\+2t[8s(Aa)]\\+t[8s(aa)]\end{array}$	$16p^4q^4$ $\begin{array}{l}2t[4s(AA)]\\+2t[4s(Aa)]\end{array}+4t[4s(Aa)]$ $+\qquad+$ $\begin{array}{l}t[4s(AA)]\\+2t[4s(Aa)]\\+t[4s(aa)]\end{array}\quad\begin{array}{l}2t[4s(Aa)]\\+2t[4s(aa)]\end{array}$	$4p^3q^5$ $4t[8s(Aa)]$ $+$ $\begin{array}{l}2t[8s(Aa)]\\+2t[8s(aa)]\end{array}$
$4p^2q^2\left\{\begin{array}{l}4s(AA)\\+\\8s(Aa)\\+\\4s(aa)\end{array}\right.$	$4p^6q^2$ $4t[4s(AA)]$ $+$ $\begin{array}{l}2t[8s(AA)]\\+2t[8s(Aa)]\end{array}$ $+$ $4t[4s(Aa)]$	$16p^5q^3$ $4t[2s(AA)]+\begin{array}{l}2t[2s(AA)]\\+2t[2s(Aa)]\end{array}$ $+\qquad+$ $\begin{array}{l}2t[4s(AA)]\\+2t[4s(Aa)]\end{array}\quad\begin{array}{l}t[4s(AA)]\\+2t[4s(Aa)]\\+t[4s(aa)]\end{array}$ $+\qquad+$ $4t[2s(Aa)]\quad\begin{array}{l}2t[2s(Aa)]\\+2t[2s(aa)]\end{array}$	$16p^4q^4$ $4t[s(AA)]+\begin{array}{l}2t[2s(AA)]\\+2t[2s(Aa)]\end{array}+4t[s(Aa)]$ $+\qquad+\qquad+$ $\begin{array}{l}2t[2s(AA)]\\+2t[2s(Aa)]\end{array}\quad\begin{array}{l}t[4s(AA)]\\+2t[4s(Aa)]\\+t[4s(aa)]\end{array}\quad\begin{array}{l}2t[2s(Aa)]\\+2t[2s(aa)]\end{array}$ $+\qquad+\qquad+$ $4t[s(Aa)]\quad\begin{array}{l}2t[2s(Aa)]\\+2t[2s(aa)]\end{array}\quad4t[s(aa)]$	$8p^4q^4$ $\begin{array}{l}2t[4s(AA)]\\+2t[4s(Aa)]\end{array}$ $+$ $\begin{array}{l}t[8s(AA)]\\+2t[8s(Aa)]\\+t[8s(aa)]\end{array}$ $+$ $\begin{array}{l}2t[4s(Aa)]\\+2t[4s(aa)]\end{array}$	$16p^3q^5$ $\begin{array}{l}2t[2s(AA)]\\+2t[2s(Aa)]\end{array}+4t[2s(Aa)]$ $+\qquad+$ $\begin{array}{l}t[4s(AA)]\\+2t[4s(Aa)]\\+t[4s(aa)]\end{array}\quad\begin{array}{l}2t[4s(Aa)]\\+2t[4s(aa)]\end{array}$ $+\qquad+$ $\begin{array}{l}2t[2s(Aa)]\\+2t[2s(aa)]\end{array}\quad4t[2s(aa)]$	$4p^2q^6$ $4t[4s(Aa)]$ $+$ $\begin{array}{l}2t[8s(Aa)]\\+2t[8s(aa)]\end{array}$ $+$ $4t[4s(aa)]$
$2p^2q^2\{16s(Aa)$	$2p^6q^2$ $\begin{array}{l}2t[16s(AA)]\\+2t[16s(Aa)]\end{array}$	$8p^5q^3$ $\begin{array}{l}2t[8s(AA)]\\+2t[8s(Aa)]\end{array}+\begin{array}{l}t[8s(AA)]\\+2t[8s(Aa)]\\+t[8s(aa)]\end{array}$	$8p^4q^4$ $\begin{array}{l}2t(4s(AA)]\\+2t(4s(Aa)]\end{array}+\begin{array}{l}t[8s(AA)]\\+2t[8s(Aa)]\\+t[8s(aa)]\end{array}+\begin{array}{l}2t[4s(Aa)]\\+2t[4s(aa)]\end{array}$	$4p^4q^4$ $\begin{array}{l}t[16s(AA)]\\+2t[16s(Aa)]\\+t[16s(aa)]\end{array}$	$8p^3q^5$ $\begin{array}{l}t[8s(AA)]\\+2t[8s(Aa)]\\+t[8s(aa)]\end{array}+\begin{array}{l}2t[8s(Aa)]\\+2t[8s(aa)]\end{array}$	$2p^2q^6$ $\begin{array}{l}2t[16s(Aa)]\\+2t[16s(aa)]\end{array}$
$4pq^3\left\{\begin{array}{l}8s(Aa)\\+\\8s(aa)\end{array}\right.$	$4p^5q^3$ $\begin{array}{l}2t[8s(AA)]\\+2t[8s(Aa)]\end{array}$ $+$ $4t[8s(Aa)]$	$16p^4q^4$ $\begin{array}{l}2t[4s(AA)]\\+2t[4s(Aa)]\end{array}+\begin{array}{l}t[4s(AA)]\\+2t[4s(Aa)]\\+t[4s(aa)]\end{array}$ $+\qquad+$ $4t[4s(Aa)]\quad\begin{array}{l}2t[4s(Aa)]\\+2t[4s(aa)]\end{array}$	$16p^3q^5$ $\begin{array}{l}2t[2s(AA)]\\+2t[2s(Aa)]\end{array}+\begin{array}{l}t[4s(AA)]\\+2t[4s(Aa)]\\+t[4s(aa)]\end{array}+\begin{array}{l}2t[2s(Aa)]\\+2t[2s(aa)]\end{array}$ $+\qquad+\qquad+$ $4t[2s(Aa)]\quad\begin{array}{l}2t[4s(Aa)]\\+2t[4s(aa)]\end{array}\quad4t[2s(aa)]$	$8p^3q^5$ $\begin{array}{l}t[8s(AA)]\\+2t[8s(Aa)]\\+t[8s(aa)]\end{array}$ $+$ $\begin{array}{l}2t[8s(Aa)]\\+2t[8s(aa)]\end{array}$	$16p^2q^6$ $\begin{array}{l}t[4s(AA)]\\+2t[4s(Aa)]\\+t[4s(aa)]\end{array}+\begin{array}{l}2t[4s(Aa)]\\+2t[4s(aa)]\end{array}$ $+\qquad+$ $\begin{array}{l}2t[4s(Aa)]\\+2t[4s(aa)]\end{array}\quad4t[4s(aa)]$	$4pq^7$ $\begin{array}{l}2t[8s(Aa)]\\+2t[8s(aa)]\end{array}$ $+$ $4t[8s(aa)]$
$q^4\{16s(aa)$	p^4q^4 $4t[16s(Aa)]$	$4p^3q^5$ $4t[8s(Aa)]+\begin{array}{l}2t[8s(Aa)]\\+2t[8s(aa)]\end{array}$	$4p^2q^6$ $4t[4s(Aa)]+\begin{array}{l}2t[8s(Aa)]\\+2t[8s(aa)]\end{array}+4t[4s(aa)]$	$2p^2q^6$ $\begin{array}{l}2t[16s(Aa)]\\+2t[16s(aa)]\end{array}$	$4pq^7$ $\begin{array}{l}2t[8s(Aa)]\\+2t[8s(aa)]\end{array}+4t[8s(aa)]$	q^8 $4t[16s(aa)]$.

to the particular constituent, protogenic, hybrid, or allogenic, each member of the pair possesses. It will be more convenient, however, to make use of the fact that the number of pairs of uncle and nephew will be equal to the number of pairs obtained by taking any one of the children from given parents with any one of the grandchildren, minus the number of pairs of parents and offspring among such pairs. This latter can be obtained more simply than by Table IV. For Table I enables us to pick out parent and offspring in pairs and arrange them according as each member of the pair is (AA), (A*a*), or (*aa*). But we require the number of such pairs in the next generation. Allowing for the change in the fertility from $16s$ to $4t$, it is clear that the number in the second generation will be obtained from the number in the first by writing $4t$ for $16s$ and multiplying by $16s\,(p+q)^4$. From Table I we can at once pick out the following terms for the first generation.

Parent (AA), offspring (AA)...... $p^4 . 16s+2p^3q . 8s$,
„ (AA), „ (A*a*)...... $2p^3q . 8s+p^2q^2 . 16s$,
„ (AA), „ (*aa*)...... 0,
„ (A*a*), „ (AA)...... $2p^3q . 8s+4p^2q^2 . 4s$,
„ (A*a*), „ (A*a*)...... $2p^3q . 8s+4p^2q^2 . 8s+2pq^3 . 8s$,
„ (A*a*), „ (*aa*)...... $4p^2q^2 . 4s+2pq^3 . 8s$,
„ (*aa*), „ (AA)...... 0,
„ (*aa*), „ (A*a*)...... $p^2q^2 . 16s+2pq^3 . 8s$,
„ (*aa*), „ (*aa*)...... $2pq^3 . 8s+q^4 . 16s$.

In obtaining these expressions, one parent only was considered. From the symmetry of the table, if both parents are to be taken into account, each term must be multiplied by two. Since this factor will occur throughout the work, it can be omitted.

Collecting the above terms, and applying the modification necessary to transform the first generation into the second, we have, for this latter generation, the frequencies:—

Parent (AA), offspring (AA)...... $64stp^3(p+q)^5$,
„ (AA), „ (A*a*)...... $64stp^2q\,(p+q)^5$,
„ (AA), „ (*aa*)...... 0,
„ (A*a*), „ (AA)...... $64stp^2q\,(p+q)^5$,
„ (A*a*), „ (A*a*)...... $64stpq\,(p+q)^6$,
„ (A*a*), „ (*aa*)...... $64stpq^2\,(p+q)^5$,
„ (*aa*), „ (AA)...... 0,
„ (*aa*), „ (A*a*)...... $64stpq^2\,(p+q)^5$,
„ (*aa*), „ (*aa*)...... $64st\,q^3\,(p+q)^5$.

From Table IV we have now to pick out and arrange the various pairs which can be obtained by taking any one in the first generation with any one of the same stock in the second generation (*i.e.* both sons and nephews).

To economise space, the details of the terms are given in the case of three only of the nine pairs. The other six can be written out in the same manner, but the results alone are stated. The three pairs given in detail are:—

Member of the first generation (AA), member of the second generation (AA)—

$$\begin{aligned}
&p^8 \cdot 16s \cdot 4t \cdot 16s + 4p^7q \cdot 16s \cdot (4t \cdot 8s + 2t \cdot 8s)\\
&+4p^6q^2 \cdot 16s\,(4t \cdot 4s + 2t \cdot 8s) + 2p^6q^2 \cdot 16s \cdot 2t \cdot 16s\\
&+4p^5q^3 \cdot 16s \cdot 2t \cdot 8s + 4p^7q \cdot 8s\,(4t \cdot 8s + 2t \cdot 8s)\\
&+16p^6q^2 \cdot 8s\,(4t \cdot 4s + 2t \cdot 4s + 2t \cdot 4s + t \cdot 4s)\\
&+16p^5q^3 \cdot 8s\,(4t \cdot 2s + 2t \cdot 4s + 2t \cdot 2s + t \cdot 4s)\\
&+8p^5q^3 \cdot 8s\,(2t \cdot 8s + t \cdot 8s) + 16p^4q^4 \cdot 8s\,(2t \cdot 4s + t \cdot 4s)\\
&+4p^6q^2 \cdot 4s\,(4t \cdot 4s + 2t \cdot 8s) + 16p^5q^3 \cdot 4s\,(4t \cdot 2s + 2t \cdot 4s + 2t \cdot 2s + t \cdot 4s)\\
&+16p^4q^4 \cdot 4s\,(4t \cdot s + 2t \cdot 2s + 2t \cdot 2s + t \cdot 4s)\\
&+8p^4q^4 \cdot 4s\,(2t \cdot 4s + t \cdot 8s) + 16p^3q^5 \cdot 4s\,(2t \cdot 2s + t \cdot 4s).
\end{aligned}$$

When these terms are collected, and simplification performed, the whole expression reduces to the simple form

$$512s^2tp^3(p+q)^4(2p+q).$$

Member of first generation (AA), member of second generation (A*a*)—

$$\begin{aligned}
&4p^7q \cdot 16s \cdot 2t \cdot 8s + 4p^6q^2 \cdot 16s\,(2t \cdot 8s + 4t \cdot 4s) + 2p^6q^2 \cdot 16s \cdot 2t \cdot 16s\\
&+4p^5q^3 \cdot 16s\,(2t \cdot 8s + 4t \cdot 8s) + p^4q^4 \cdot 16s \cdot 4t \cdot 16s\\
&+4p^7q \cdot 8s \cdot 2t \cdot 8s + 16p^6q^2 \cdot 8s\,(2t \cdot 4s + 2t \cdot 4s + 2t \cdot 4s)\\
&+16p^5q^3 \cdot 8s\,(2t \cdot 2s + 2t \cdot 4s + 2t \cdot 4s + 4t \cdot 2s + 2t \cdot 2s)\\
&+8p^5q^3 \cdot 8s\,(2t \cdot 8s + 2t \cdot 8s) + 16p^4q^4 \cdot 8s\,(2t \cdot 4s + 2t \cdot 4s + 4t \cdot 4s + 2t \cdot 4s)\\
&+4p^3q^5 \cdot 8s\,(4t \cdot 8s + 2t \cdot 8s) + 4p^6q^2 \cdot 4s\,(2t \cdot 8s + 4t \cdot 4s)\\
&+16p^5q^3 \cdot 4s\,(2t \cdot 4s + 4t \cdot 2s + 2t \cdot 2s + 2t \cdot 4s + 2t \cdot 2s)\\
&+16p^4q^4 \cdot 4s\,(2t \cdot 2s + 4t \cdot s + 2t \cdot 2s + 2t \cdot 4s + 2t \cdot 2s + 4t \cdot s + 2t \cdot 2s)\\
&+8p^4q^4 \cdot 4s\,(2t \cdot 4s + 2t \cdot 8s + 2t \cdot 4s)\\
&+16p^3q^5 \cdot 4s\,(2t \cdot 2s + 2t \cdot 4s + 2t \cdot 2s + 4t \cdot 2s + 2t \cdot 4s)\\
&+4p^2q^6 \cdot 4s\,(4t \cdot 4s + 2t \cdot 8s).
\end{aligned}$$

This, when simplified, becomes

$$512s^2tp^2q\,(p+q)^4(3p+q).$$

Member of first generation (AA), member of second generation (*aa*)—

$$16p^6q^2\,.\,8s\,.\,t\,.\,4s+16p^5q^3\,.\,8s\,(t\,.\,4s+2t\,.\,2s)+8p^5q^3\,.\,8s\,.\,t\,.\,8s$$
$$+16p^4q^4\,.\,8s\,(t\,.\,4s+2t\,.\,4s)+4p^3q^5\,.\,8s\,.\,2t\,.\,8s$$
$$+16p^5q^3\,.\,4s\,(t\,.\,4s+2t\,.\,2s)+16p^4q^4\,.\,4s\,(t\,.\,4s+2t\,.\,2s+2t\,.\,2s+4t\,.\,s)$$
$$+8p^4q^4\,.\,4s\,(t\,.\,8s+2t\,.\,4s)+16p^3q^5\,.\,4s\,(t\,.\,4s+2t\,.\,2s+2t\,.\,4s+4t\,.\,2s)$$
$$+4p^2q^6\,.\,4s\,(2t\,.\,8s+4t\,.\,4s).$$

This reduces to $512s^2t\,p^2q^2\,(p+q)^4$.

The frequencies of the other pairs are (the member of the first generation in each case being placed first)

(A*a*)	with	(AA)......	$512s^2tp^2q\,(p+q)^4\,(3p+q)$,
(A*a*)	„	(A*a*)......	$512s^2tpq\,(p+q)^4\,(p^2+6pq+q^2)$,
(A*a*)	„	(*aa*)......	$512s^2tpq^2\,(p+q)^4\,(p+3q)$,
(*aa*)	„	(AA)......	$512s^2tp^2q^2\,(p+q)^4$,
(*aa*)	„	(A*a*)......	$512s^2tpq^2\,(p+q)^4\,(p+3q)$,
(*aa*)	„	(*aa*)......	$512s^2t\,q^3\,(p+q)^4\,(p+2q)$.

Subtracting from these expressions those found for the corresponding combinations in the case of parent and offspring of the second generation given above, we get the following frequencies for combinations of uncle and nephew :—

Uncle	(AA),	nephew	(AA)...	$64stp^3\,(p+q)^4\,[(16s-1)p+(8s-1)\,q]$,
„	(AA),	„	(A*a*)...	$64stpq\,(p+q)^4\,[(24s-1)p^2+(8s-1)pq]$,
„	(AA),	„	(*aa*)...	$512s^2tp^2q^2\,(p+q)^4$.
„	(A*a*),	„	(AA)...	$64stpq\,(p+q)^4\,[(24s-1)p^2+(8s-1)pq]$,
„	(A*a*),	„	(A*a*)...	$64stpq\,(p+q)^4\,[(8s-1)p^2+(48s-2)pq+(8s-1)\,q^2]$,
„	(A*a*),	„	(*aa*)...	$64stpq^2\,(p+q)^4\,[(8s-1)p+(24s-1)\,q]$,
„	(*aa*),	„	(AA)...	$512s^2tp^2q^2\,(p+q)^4$,
„	(*aa*),	„	(A*a*)...	$64stpq^2\,(p+q)^4\,[(8s-1)p+(24s-1)\,q]$,
„	(*aa*),	„	(*aa*)...	$64st\,q^3\,(p+q)^4\,[(8s-1)p+(16s-1)\,q]$.

Correlation tables can now be formed from which to derive the gametic and the somatic relationships between uncle and nephew. Table V gives the *gametic* relationship; Table VI the *somatic*. In both of these tables the factor $64\,st\,(p+q)^4$, which multiplies every cell, has been omitted.

Table V.—Gametic Correlation between Uncle and Nephew.

(Factor $64st(p+q)^4$ omitted throughout.)

Nephew.	Uncle.			Totals.
	(AA.)	(A*a*.)	(*aa*.)	
(AA)	$(16s-1)p^4+(8s-1)p^3q$	$(24s-1)p^3q+(8s-1)p^2q^2$	$8sp^2q^2$	$(16s-1)p^2(p+q)^2$
(A*a*)	$(24s-1)p^3q+(8s-1)p^2q^2$	$(8s-1)p^3q+(48s-2)p^2q^2$ $+(8s-1)pq^3$	$(8s-1)p^2q^2+(24s-1)pq^3$	$2(16s-1)pq(p+q)^2$
(*aa*)	$8sp^2q^2$	$(8-s1)p^2q^2+(24s-1)pq^3$	$(8s-1)pq^3+(16s-1)q^4$	$(16s-1)q^2(p+q)^2$
Totals	$(16s-1)p^2(p+q)^2$	$2(16s-1)pq(p+q)^2$	$(16s-1)q^2(p+q)^2$	$(16s-1)(p+q)^4$

Table VI.—Somatic Correlation between Uncle and Nephew.

(Factor $64st(p+q)^4$ omitted throughout.)

Nephew.	Uncle.		Totals.
	(A.)	(Not A.)	
(A)	$(16s-1)p^4+4(16s-1)p^3q+4(16s-1)p^2q^2$ $+(8s-1)pq^3$	$(16s-1)p^2q^2+(24s-1)pq^3$	$(16s-1)p(p+q)^2(p+2q)$
(Not A)	$(16s-1)p^2q^2+(24s-1)pq^3$	$(8s-1)pq^3+(16s-1)q^4$	$(16s-1)q^2(p+q)^2$
Totals	$(16s-1)p(p+q)^2(p+2q)$	$(16s-1)q^2(p+q)^2$	$(16s-1)(p+q)^4$

(14) *Gametic Correlation.*—From Table V we find, measuring from the centre of the middle row—

$$\bar{y}_1 = \frac{(16s-1)p^2+(8s-1)pq-8sq^2}{(16s-1)(p+q)^2} \qquad \bar{y}_3 = \frac{8sp^2-(8s-1)pq-(16s-1)q^2}{(16s-1)(p+q)^2},$$

$$\bar{y}_2 = \frac{(24s-1)(p^2-q^2)}{2(16s-1)(p+q)^2} \qquad \bar{y} = \frac{p^2-q^2}{(p+q)^2} = \frac{p-q}{p+q}.$$

Hence
$$\bar{y}_1-\bar{y}_2 = \frac{8s-1}{16s-1}\cdot\frac{1}{2} = \bar{y}_2-\bar{y}_3 = r.$$

Therefore the regression is linear and the correlation $\frac{8s-1}{16s-1}\cdot\frac{1}{2}$. As before, we must take a random sample from the families obtained by making s indefinitely large. The correlation then becomes $\frac{1}{4}$.

(15) *Somatic Correlation.*—Table VI gives, measuring from the "not A" row—

$$\bar{y} = \frac{p(p+2q)}{(p+q)^2}$$

and

$$\bar{y}_1 - \bar{y}_2 = \frac{8s-1}{16s-1} \cdot \frac{q}{p+2q} = r.$$

Again the correlation depends upon the relative values of p and q. The limits between which r can lie for different values of p and q are 0 and $\frac{8s-1}{16s-1} \cdot \frac{1}{2}$. If $p = q$, its value is $\frac{8s-1}{16s-1} \cdot \frac{1}{3}$. Putting s infinite, the range for r is from 0 to $\frac{1}{4}$, with the value $\frac{1}{6}$ when $p = q$.

(C.) *Correlation between First Cousins.*

(16) From Table IV we can pick out every pair of first cousins, and arrange them in groups according as each member of the pair exhibits the constituent (AA), (A*a*) or (*aa*). But, again, it is more convenient to obtain these groups indirectly. For the number of pairs of cousins in any group will be equal to the number of pairs which can be taken from all children having the same two grandparents, minus the number of pairs of brothers among such children. Since the population is stable during succeeding generations, the latter can be obtained at once from the expressions given in (7) by writing $4t$ for $16s$ in that table and multiplying by the factor $16s(p+q)^4$. We therefore have the following expressions for the number of pairs of brothers among all grandchildren :—

First brother.	Second brother.	
(AA)	(AA)......	$32st(p+q)^4[(4t-1)p^4+(4t-2)p^3q+(t-1)p^2q^2]$,
(AA)	(A*a*)......	$64st(p+q)^4[4tp^3q+2tp^2q^2]$,
(AA)	(*aa*)	$64st(p+q)^4 tp^2q^2$,
(A*a*)	(A*a*)......	$32st(p+q)^4[(4t-2)p^3q+4(3t-1)p^2q^2+(4t-2)pq^3]$,
(A*a*)	(*aa*)	$64st(p+q)^4[2tp^2q^2+4tpq^3]$,
(*aa*)	(*aa*)	$32st(p+q)^4[(t-1)p^2q^2+(4t-2)pq^3+(4t-1)q^4]$.

The number of pairs of all possible grandchildren (*i.e.* siblings, together with cousins) can be easily obtained from inspection of Table IV. From symmetry, only six combinations are now required. Three of these are given in detail, the final expressions only of the other three being written down. Throughout the detailed expressions k is written for the product st.

First grandchild (AA), second grandchild (AA)—

$$
\begin{aligned}
&p^8 \cdot \tfrac{1}{2} \cdot 64k(64k-1)+4p^7q \cdot \tfrac{1}{2} \cdot 48k(48k-1)+4p^6q^2 \cdot \tfrac{1}{2} \cdot 32k(32k-1)\\
&+2p^6q^2 \cdot \tfrac{1}{2} \cdot 32k(32k-1)+4p^5q^3 \cdot \tfrac{1}{2} \cdot 16k(16k-1)+4p^7q \cdot \tfrac{1}{2} \cdot 48k(48k-1)\\
&+16p^6q^2 \cdot \tfrac{1}{2} \cdot 36k(36k-1)+16p^5q^3 \cdot \tfrac{1}{2} \cdot 24k(24k-1)+8p^5q^3 \cdot \tfrac{1}{2} \cdot 24k(24k-1)\\
&+16p^4q^4 \cdot \tfrac{1}{2} \cdot 12k(12k-1)+4p^6q^2 \cdot \tfrac{1}{2} \cdot 32k(32k-1)+16p^5q^3 \cdot \tfrac{1}{2} \cdot 24k(24k-1)\\
&+16p^4q^4 \cdot \tfrac{1}{2} \cdot 16k(16k-1)+8p^4q^4 \cdot \tfrac{1}{2} \cdot 16k(16k-1)+16p^3q^5 \cdot \tfrac{1}{2} \cdot 8k(8k-1)\\
&+2p^6q^2 \cdot \tfrac{1}{2} \cdot 32k(32k-1)+8p^5q^3 \cdot \tfrac{1}{2} \cdot 24k(24k-1)+8p^4q^4 \cdot \tfrac{1}{2} \cdot 16k(16k-1)\\
&+4p^4q^4 \cdot \tfrac{1}{2} \cdot 16k(16k-1)+8p^3q^5 \cdot \tfrac{1}{2} \cdot 8k(8k-1)+4p^5q^3 \cdot \tfrac{1}{2} \cdot 16k(16k-1)\\
&+16p^4q^4 \cdot \tfrac{1}{2} \cdot 12k(12k-1)+16p^3q^5 \cdot \tfrac{1}{2} \cdot 8k(8k-1)+8p^3q^5 \cdot \tfrac{1}{2} \cdot 8k(8k-1)\\
&+16p^2q^6 \cdot \tfrac{1}{2} \cdot 4k(4k-1).
\end{aligned}
$$

This reduces to

$$32stp^2(p+q)^4[(64st-1)p^2+(32st-2)pq+(4st-1)q^2].$$

First grandchild (AA), second grandchild (A*a*)—

$$
\begin{aligned}
&4p^7q \cdot 48k \cdot 16k+4p^6q^2 \cdot 32k \cdot 32k+2p^6q^2 \cdot 32k \cdot 32k\\
&+4p^5q^3 \cdot 16k \cdot 48k+4p^7q \cdot 48k \cdot 16k+16p^6q^2 \cdot 36k \cdot 24k\\
&+16p^5q^3 \cdot 24k \cdot 32k+8p^5q^3 \cdot 24k \cdot 32k+16p^4q^4 \cdot 12k \cdot 40k\\
&+4p^6q^2 \cdot 32k \cdot 32k+16p^5q^3 \cdot 24k \cdot 32k+16p^4q^4 \cdot 16k \cdot 32k\\
&+8p^4q^4 \cdot 16k \cdot 32k+16p^3q^5 \cdot 8k \cdot 32k+2p^6q^2 \cdot 32k \cdot 32k\\
&+8p^5q^3 \cdot 24k \cdot 32k+8p^4q^4 \cdot 16k \cdot 32k+4p^4q^4 \cdot 16k \cdot 32k\\
&+8p^3q^5 \cdot 8k \cdot 32k+4p^5q^3 \cdot 16k \cdot 48k+16p^4q^4 \cdot 12k \cdot 40k\\
&+16p^3q^5 \cdot 8k \cdot 32k+8p^3q^5 \cdot 8k \cdot 32k+16p^2q^6 \cdot 4k \cdot 24k.
\end{aligned}
$$

This becomes $512s^2t^2p^2q(p+q)^4(12p+3q).$

First grandchild (AA), second grandchild (*aa*)—

$$
\begin{aligned}
&16p^6q^2 \cdot 36k \cdot 4k+16p^5q^3 \cdot 24k \cdot 8k+8p^5q^3 \cdot 24k \cdot 8k\\
&+16p^4q^4 \cdot 12k \cdot 12k+16p^5q^3 \cdot 24k \cdot 8k\\
&+16p^4q^4 \cdot 16k \cdot 16k+8p^4q^4 \cdot 16k \cdot 16k+16p^3q^5 \cdot 8k \cdot 24k\\
&+8p^5q^3 \cdot 24k \cdot 8k+8p^4q^4 \cdot 16k \cdot 16k+4p^4q^4 \cdot 16k \cdot 16k\\
&+8p^3q^5 \cdot 8k \cdot 24k+16p^4q^4 \cdot 12k \cdot 12k+16p^3q^5 \cdot 8k \cdot 24k\\
&+8p^3q^5 \cdot 8k \cdot 24k+16p^2q^6 \cdot 4k \cdot 36k.
\end{aligned}
$$

This becomes $2304s^2t^2p^2q^2(p+q)^4.$

The other combinations reduce to:—

First grandchild (A*a*), second grandchild (A*a*)—

$$64stpq(p+q)^4[(16st-1)p^2+(104st-2)pq+(16st-1)q^2].$$

First grandchild (Aa), second grandchild (aa)—

$$512s^2t^2pq^2(p+q)^4(3p+12q).$$

First grandchild (aa), second grandchild (aa)—

$$32st\,q^2(p+q)^4[(4st-1)p^2+(32st-2)pq+(64st-1)q^2].$$

To obtain the frequencies of the various pairs of cousins, we have to subtract from the above six expressions the corresponding expressions for pairs of brothers. We then get the following frequencies:—

Both cousins (AA)—$32\,st^2p^2(p+q)^4[64s-4)p^2+(32s-4)pq+(4s-1)q^2]$.

One cousin (AA), other (Aa)... $128st^2p^2q(p+q)^4[(48s-2)p+(12s-1)q]$.

" (AA), " (aa) ... $64st^2p^2q^2(p+q)^4(36s-1)$.

Both cousins (Aa)—

$$128st^2pq(p+q)^4[(8s-1)p^2+(52s-3)pq+(8s-1)q^2].$$

One cousin (Aa), other (aa)—

$$128st^2pq^2(p+q)^4[(12s-1)p+(48s-2)q].$$

Both cousins (aa)—

$$32st^2q^2(p+q)^4[(4s-1)p^2+(32s-4)pq+(64s-4)q^2].$$

The correlations tables for cousins can now be formed. Table VII gives the arrangement for the *gametic* relationship, Table VIII for the *somatic*. In both cases the factor $64\,st^2(p+q)^4$ has been omitted. The tables must, of course, be made symmetrical.

Table VII.—Gametic Correlation between Cousins.
(Factor $64\,st^2(p+q)^4$ omitted.)

Second cousin.	First cousin.			Totals.
	(AA).	(Aa).	(aa).	
(AA)	$(64s-4)p^4+(32s-4)p^3q$ $+(4s-1)p^2q^2$	$(96s-4)p^3q+(24s-2)p^2q^2$	$(36s-1)p^2q^2$	$4(16s-1)p^2(p+q)^2$
(Aa)	$(96s-4)p^3q+(24s-2)p^2q^2$	$(32s-4)p^3q$ $+(208s-12)p^2q^2$ $+(32s-4)pq^3$	$(24s-2)p^2q^2+(96s-4)pq^3$	$8(16s-1)pq(p+q)^2$
(aa)	$(36s-1)p^2q^2$	$(24s-2)p^2q^2+(96s-4)pq^3$	$(4s-1)p^2q^2+(32s-4)pq^3$ $+(64s-4)q^4$	$4(16s-1)q^2(p+q)^2$
Totals	$4(16s-1)p^2(p+q)^2$	$8(16s-1)pq(p+q)^2$	$4(16s-1)q^2(p+q)^2$	$4(16s-1)(p+q)^4$

Table VIII.—Somatic Correlation between Cousins.
(Factor $64st^2(p+q)^4$ omitted.)

Second cousin.	First cousin.		Totals.
	(A).	(Not A).	
(A)	$4(16s-1)p^4+16(16s-1)p^3q$ $+(260s-17)p^2q^2+4(8s-1)pq^3$	$4(24s-1)pq^3+3(20s-1)p^2q^2$	$4(16s-1)(p+q)^2p(p+2q)$
(Not A)	$4(24s-1)pq^3+3(20s-1)p^2q^2$	$4(16s-1)q^4+4(8s-1)pq^3$ $+(4s-1)p^2q^2$	$4(16s-1)q^2(p+q)^2$
Totals	$4(16s-1)(p+q)^2p(p+2q)$	$4(16s-1)q^2(p+q)^2$	$4(16s-1)(p+q)^4$

(17) *Gametic Correlation.*—From Table VII we find, after simplification—

$$\bar{y}_1 = \frac{(16s-1)p-8sq}{(16s-1)(p+q)} \qquad \bar{y}_3 = \frac{8sp-(16s-1)q}{(16s-1)(p+q)}$$

$$\bar{y}_2 = \frac{(24s-1)(p-q)}{2(16s-1)(p+q)} \qquad \bar{y} = \frac{p-q}{p+q}.$$

From these, $\bar{y}_1-\bar{y}_2 = \dfrac{8s-1}{2(16s-1)} = \bar{y}_2-\bar{y}_3 = r$. Hence the regression again is linear, and the correlation $\dfrac{8s-1}{16s-1}\cdot\dfrac{1}{2}$. When s is indefinitely large this becomes $\frac{1}{4}$.

(18) *Somatic Correlation.*—In this case, after reduction, we find—

$$\bar{y} = \frac{p(p+2q)}{(p+q)^2} \quad \text{and} \quad \bar{y}_1-\bar{y}_2 = \frac{(4s-1)p+4(8s-1)q}{4(16s-1)(p+2q)} = r.$$

Hence in the case of cousins also the *somatic* correlation depends upon the ratio of p to q. The range of r for different values of p and q extends from $\dfrac{4s-1}{4(16s-1)}$ to $\dfrac{8s-1}{2(16s-1)}$. If $p = q$ its value is $\dfrac{36s-5}{12(16s-1)}$. When s is indefinitely large, the range is from $\frac{1}{16}$ to $\frac{1}{4}$, with the value $\frac{3}{16}$ when $p = q$.

(19) In Table IX, all the correlations which have been worked out from the Mendelian hypothesis of "unit characters" are collected together. The values of the parental and grandparental correlations are from Prof. Pearson's paper.* The rest have been obtained in the present paper.

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* "On the Ancestral Correlations of a Mendelian Population," 'Roy. Soc. Proc.,' B, 1909, vol. 81.

Table IX.—List of *Mendelian* Correlations between an Individual and his Relatives.

Relative.	Gametic correlation.	Somatic correlation.		
		General.	Range.	$p = q$.
Parent	$\frac{1}{2}$	$\frac{q}{p+2q}$	$0-\frac{1}{2}$	$\frac{1}{3}$
Grandparent	$\frac{1}{4}$	$\frac{q}{p+2q} \cdot \frac{1}{2}$	$0-\frac{1}{4}$	$\frac{1}{6}$
Brother or sister	$\frac{1}{2}$	$\frac{p+4q}{p+2q} \cdot \frac{1}{4}$	$\frac{1}{4}-\frac{1}{2}$	$\frac{5}{12}$
Uncle or aunt	$\frac{1}{4}$	$\frac{q}{p+2q} \cdot \frac{1}{2}$	$0-\frac{1}{4}$	$\frac{1}{6}$
Cousin	$\frac{1}{4}$	$\frac{p+8q}{p+2q} \cdot \frac{1}{16}$	$\frac{1}{16}-\frac{1}{4}$	$\frac{3}{16}$

From this table we notice that, on the Mendelian hypothesis, the parental and sibling gametic correlations are equal, while the grandparental, avuncular, and cousin gametic correlations are also equal and are just one-half of the former. In Table X the ancestral and collateral correlations in man and certain animals founded on biometric research, together with the corresponding *gametic* correlations found above on the Mendelian assumption of "unit-characters," are collected.

(20) The following general conclusions can be drawn from the correlations for *somatic* characters given in Table IX.

(i) If $p > 8q$ the cousin correlation is greater than the parental. This seems remarkable, but it must be borne in mind that the condition is derived from the Mendelian idea of "unit-characters," and no prediction can be made as to when the condition is fulfilled. The only cousin correlations worked out for the biometric school are those by Miss Elderton. No correlation for a single character in cousins was found to exceed the parental one. In eye-colour alone did the former approach the latter. The parental correlation in this case was 0·495, while the cousin correlation had the values 0·44, 0·48, and 0·38 for male and male, male and female, and female and female respectively, the mean value being 0·434.

(ii) If q is small, *i.e.* if the recedent constituent is rare, as in the case of albinism in man, the correlations founded on Mendelism have low values, and in certain cases approach zero. For pathological characters the figures found by Miss Elderton were certainly much smaller than for other characters in

Table X.—Mean Ancestral and Collateral Correlations in Man and Animals.*

—	Parent.	Grand-parent.	Sibling.	Uncle.	Cousin.
Man	0·470	0·317	0·521	0·265	0·267
Horse	0·522	0·296			
Basset hound	0·526	0·220	0·508		
Greyhound	0·532	0·332	0·559		
Shorthorn	0·444	0·200	0·530		
Mean of biometric results	0·498	0·273	0·529	0·265	0·267
Gametic correlation on Mendelian hypothesis.	0·500	0·250	0·500	0·250	0·250

cousins, but this was ascribed to the incompleteness of the records. No general tendency in the direction of lower correlations for certain characters has yet been noticed in biometric investigations.

(iii) The correlation between siblings is always greater than that between parent and offspring. This is in accordance with *a priori* expectation (see para. 3 above) and agrees with statistical results.

(iv) The grandparental correlation is always the same as the avuncular. This also agrees fairly well with statistical conclusions.

In his paper of last year Prof. Pearson drew attention to the fact that the values of the ancestral *gametic* correlations in a Mendelian population obtained from theoretical considerations were in close accordance with those determined for the *somatic* characters in biometric researches, while the *somatic* correlation on the Mendelian hypothesis appeared to be too low, if the principle of absolute dominance was maintained. The results of the present paper for the sibling, avuncular and cousin correlations agree equally well with the statement made for ancestral correlations. The general *somatic* correlation for cousins, viz., $\frac{1}{16}\frac{p+8q}{p+2q}$, is always greater than the general *somatic* grandparental and avuncular correlations. The conclusion of Miss Elderton that "cousins must be classed as equally important with uncles and aunts, and they may eventually turn out to be as important as grandparents," paradoxical as it appears, is supported by the theory of Mendelism as well as by biometric experience. Without making any dogmatic assertion, it seems safe to conclude, both from theory and practice, that the cousin ranks in no way behind aunt or uncle and grandparent in family resemblance. The importance of this deduction in medical diagnosis has already been indicated.

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* Each biometric correlation given in the table is the mean of a number of values and is compiled from the following sources :—

(1) Karl Pearson and Alice Lee, "On the Laws of Inheritance in Man," Parts I and II, 'Biometrika,' vols. 2 and 3.

(2) Karl Pearson, "On Inheritance of the Mental and Moral Characters in Man," *ibid.*, vol. 3.

(3) Amy Barrington, Alice Lee and Karl Pearson, "On Inheritance of Coat Colour in Greyhounds," *ibid.*, vol. 3.

(4) Amy Barrington and Karl Pearson, "On Inheritance of Coat Colour in Cattle," *ibid.*, vol. 4.

(5) Ethel M. Elderton, "On the Measure of the Resemblance of First Cousins," 'Eugenics Laboratory Memoirs,' IV, 1907.

The characters correlated for man include stature, span, forearm and eye-colour. The sibling correlations include also hair-colour, together with those of no less than 16 characters obtained from school records. The mean fraternal correlation based on this homogeneous material was 0·519. The cousin correlations were deduced from eye-colour, hair-colour, health, ability, etc. Among the animals, coat-colour was the character correlated throughout.

In conclusion I have to thank Prof. Pearson for considerable advice while the work in connection with this paper has been in progress.

The Origin of the Hydrochloric Acid in the Gastric Tubules.[*]

By Mabel Purefoy FitzGerald.

(Communicated by Prof. A. B. Macallum, F.R.S. Received June 4, 1910.)

(From the Biochemical Laboratory of the University of Toronto.)

[Plates 7—9.]

In 1823 William Prout[†] brought forward the view that the acid normally existing in the stomach was free hydrochloric acid, or to quote his own words, "free or, at least, unsaturated muriatic acid." This opinion was based on the analyses made by him of the gastric contents of the rabbit and of other animals, and of the fluid ejected from the human stomach in severe cases of dyspepsia. He said further: "With respect to the nature of this acid, very various opinions have been entertained. Some of the older chemists seem to have considered it as an acid *sui generis;* by others it was supposed to be the phosphoric, the acetic, the lactic, etc. No less various have been the opinions respecting its origin and use, some supposing that it is derived from the stomach itself, and is essential to the digestive process, others that it is derived from the food, or is a result of fermentation, etc.; in short, there seems to be no physiological subject so imperfectly understood, or concerning which there has been such a variety of opinions."

These words written in retrospection by the first exponent of the free hydrochloric acid theory, when read in the twentieth century, have the significance also of a prognostication, for during the past eighty-seven years interminable discussion has ensued between the advocates of the mineral and organic acid theories respectively, and in spite of the efforts of the physiologist, biologist, and bio-chemist in their several fields, uncertainty still exists on many and similar points. This is true in particular of the structure or structures of the gastric mucosa directly concerned with the formation and secretion of the hydrochloric acid, as well as of the existence even of hydrochloric acid in a demonstrable form within the gland tubules.

Bickel,[‡] one of the most recent writers on the subject, calls attention to this uncertainty, and says an answer is still required to the following questions:—"Wird die Salzsäure in den Belegzellen oder überhaupt in Zellen der Magenschleimhaut selbst gebildet, oder scheiden die Zellen nur das Ausgangsmaterial ab, aus dem die Salzsäure im Ausführungsgang der Drüsen oder an der Oberfläche der Schleimhaut entsteht?"

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[*] Part of this research was carried out during the tenure of a Travelling Fellowship of the Rockefeller Institute for Medical Research, New York, 1908.

[†] W. Prout, 'Phil. Trans.,' 1824, p. 45.

[‡] A. Bickel, "Magen und Magensaft," article in 'Handb. d. Biochemie d. Menschen u. Tiere.' Carl Oppenheimer, 1909, vol. 3, Part 1, p. 72.

The investigation, of which the present communication is the outcome, was undertaken (1908–9) with the hope of advancing, by means of micro-chemistry, the knowledge requisite to the solution of such questions. Before entering into details, an account must be given of some of the work of earlier investigators, which in the limits of the present paper must be confined mainly to researches definitely directed towards tracing the acid to its seat of formation by micro-chemical methods. Respecting the nature of the acid, the brief statement must suffice, that the tendency of recent researches has been to show that organic acids are only adventitiously present, and the view held to-day is that the hydrochloric acid is not entirely free.

In 1846 Frerichs* held the opinion that the gastric juice was secreted in an acid condition, and the free acid present was not of external origin. He pointed out that the acid nature of the secretion could be proved, even if the fluid found in the stomach was neutral in reaction, for, if in such cases the mucosa were scraped, the contents of the deepest part of the glands ("Labdrüsen") would be found to be acid in reaction. He also states that this fact is easily proved in the stomach ("Vormagen") of a bird, such as the goose, for in the deepest part of the glands a material is found which colours litmus red.

Claude Bernard (1850)† was the first to apply micro-chemistry to determine the precise seat of formation of the acid of the gastric juice. In his well-known Prussian blue experiment the hypothesis was that, since the presence of an acid was necessary to the formation of Prussian blue from the interaction of lactate of iron and potassium ferrocyanide, solutions of such salts, owing to the alkalinity of the blood, could be introduced into the circulation of an animal without fear of their interaction, and that a precipitation of Prussian blue would occur when the two salts came into contact with an acid fluid or tissue of the body; therefore, if acid were formed in any of the glandules of the gastric mucosa its presence would thus be revealed.

He injected consecutively into the jugular vein of a semi-fasting rabbit a solution of lactate of iron and a solution of potassium ferrocyanide, both solutions being tepid. Three-quarters of an hour later the animal was killed, and the tissues immediately examined. No blue colour was visible macroscopically until the stomach was opened. A deposit of Prussian blue was then found on the surface of the mucosa, more particularly in the region corresponding to the lesser curvature of the organ. Microscopical examination, however, furnished no evidence of the presence of a similar deposit in the gland tubules. Bernard therefore concluded that the gastric secretion did not acquire its acid properties until it reached the superficial region of the mucosa and had mixed with other fluids of the stomach.

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* F. Th. Frerichs, 'Wagner's Handwörterb. d. Physiol.,' vol. 3, Part 1, p. 780.

† C. Bernard, 'Leçons sur les Propriétés physiologiques et les Altérations pathologiques des Liquides de l'Organisme,' Paris, 1859.

That both the salts had reached the tissues, and were in a condition to allow their interaction to form Prussian blue in a suitable medium, was proved by their presence in the urine, and the instantaneous occurrence of the Prussian blue reaction on the addition to it of either hydrochloric or sulphuric acid. Other tissues of the injected rabbit were placed in an acid bath (H_2SO_4); as a result Prussian blue was found in the lymphatic glands of the upper part of the neck and in the orifices of the glands of the pharynx.

Bernard was further strengthened in his belief that the acid was formed externally to the glands and on the surface of the mucosa, by the negative results obtained in later experiments*; also, by the fact that after washing the mucosa with water, or injecting it with a continuous current of water charged with carbonate of soda, the acid reaction reappeared on the surface at the end of a certain interval of time depending on the method of procedure. This led him to the conclusion that the acidity was due to a fermentation of the mucus, and as proof he pointed out that if the non-acid mucus were removed and isolated for a short time it would become acid, a result excluding any possibility of action by the so-called acid glands. At a still later date† he regarded the acid as a remote product of the stomach secretion, holding the view that the glands secreted a liquid which split up into an acid fluid and another undetermined product, and that the appearance of the acid on the surface of the mucosa after death, occurring even after the stomach had been immersed in a dilute solution of sodium carbonate, was due to a continuation of secretion, the secretory processes continuing after death until putrefaction causes the impairment of an organ. He also mentions that the acid thus found was soluble in alcohol.

Observations on the reaction of the gastric mucosa of birds were made by Brücke‡ in 1859. Experimenting with a living pigeon, he found that the reaction of the surface of the mucosa of both the œsophagus and gizzard was alkaline, but that this was markedly acid in the region of the stomach glands.

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* 'Gaz. Heb. de Med.,' 1873, p. 691; discussion on paper by Lepine.

† C. Bernard, 'Gazette Médicale,' Paris, 1877 ('Soc. de Biol.'), pp. 224 and 261.

‡ Brücke, 'Sitz. K. Akad. Wiss.,' Vienna, vol. 37, 1859.

After quickly detaching the glands from the external coats of a portion of the stomach wall, isolated immediately after death, and pressing these between blue litmus paper, he found the reaction to be, as a rule, neutral, or, at most, faintly acid, even during full digestive activity. Nevertheless, he was of the opinion that the acid was formed in the glands, and he explains the lack of the acid reaction by supposing (1) the acid to be secreted in very small quantities, and to be rapidly and completely expelled after formation from the glandular lumina; and (2), by the neutralisation of the acid if it diffused out into the surrounding alkaline lymph.

In the stomach ("Vormagen") of the hen, which is composed of many multilobular glands—the secretion of each lobe being discharged into a central cavity and thence into a common excretory canal in communication with the stomach—the aggregation of the secretion rendered it sometimes possible to demonstrate an acid reaction to litmus paper.

In rabbits, he also found the reaction of the tubules to be neutral to litmus paper, although the secretion of the surface of the mucosa was intensely acid. Brücke also held the view that when hydrochloric acid is formed in the cells, an alkaline fluid of equal concentration must also be formed which passes into the lymph and blood of the gastric mucosa; thus the isolated glandular tissue should not give an acid reaction with litmus paper.

The recognition of the existence of two kinds of cells in the fundus glands ("Labdrüsen"), the description of which was afforded by Heidenhain* and Rollett† in quick succession, together with the suggestion made by Heidenhain of their functional difference, *i.e.* of the formation of pepsin by the chief cells (Rollett's adelomorph cells), and of the acid of the gastric juice by the parietal cells (Rollett's delomorph cells), gave renewed impetus to the investigation of the seat of the acid formation; and many micro-chemical methods were subsequently employed to determine a possible connection between the secretion of acid and the parietal cells in particular.

Rollett (1871),‡ hoping to establish such a connection between different regions or certain cells of the gastric glands ("Labdrüsen"), repeated the experiment of Claude Bernard, but used sodium ferrocyanide instead of the potassium salt, in conjunction with lactate of iron. He was unable to obtain uniform results, and although many rabbits were injected, in only two cases did he obtain a light blue tinge on the surface of the mucosa, which appeared in the transitional region of the *Portio Pylorica.*

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* Heidenhain, 'Arch. Mikros. Anat.,' 1870.

† A. Rollett, 'Centralbl. Med. Wiss.,' 1870, pp. 21, 22.

‡ A. Rollett, 'Untersuch. a. d. Inst. f. Physiol. u. Histol., Graz,' 1871, Part 2, p. 192.

Lepine (1872)* also repeated the injection experiment of Claude Bernard, and injected a dog with a solution of potassium ferrocyanide and a solution of lactate of iron by the left carotid artery, thus causing the animal's death. Like Claude Bernard, he found a deposit of Prussian blue on the surface of the gastric mucosa, but after fixing the organ in Müller's fluid, in which Prussian blue, being insoluble, is unaffected, he obtained no evidence of its occurrence in the cells.

This method failing, he altered the technique in a variety of ways, hoping by one or other process to prove the point in question. Thus, he macerated the stomach of an uninjected animal (dog) in full secretion in a solution of potassium ferrocyanide and lactate of iron; or, after death, macerated the stomach of a dog, previously injected with a solution of potassium ferrocyanide, in one of lactate of iron. Both methods gave negative results.

Later, Lepine prepared a solution in which sulphate of iron and potassium ferrocyanide were present in the proportions and manner necessary for the formation of Prussian blue, and neutralised this with dilute caustic potash (litmus paper as indicator), thus causing the blue colour to disappear and the solution to become a dirty yellow. A drop of dilute hydrochloric acid added to several cubic centimetres of the fluid was sufficient to bring back the blue colour. In this solution he placed fresh sections of the stomach of normal dogs, killed by section of the medulla, one to three hours after the ingestion of a meal given after a fast of 24 hours. The contents of the stomach and the surface of the mucosa were in each case found to be acid to litmus. In some instances, before the meal, a dilute solution of bicarbonate of soda was injected into the stomach. Lepine argued that if acid were present in the glands, even if very dilute, the blue colour would appear in the sections when placed in the neutral fluid. The tissues were left in the solution for varying lengths of time, but a blue colour did not appear.

A portion of the mucosa detached from the outer coats of the stomach was also arranged as a dialyser, with an alcoholic solution of sulphate or lactate of iron on the one side, and an aqueous solution of potassium ferrocyanide on the other. Sometimes the dialysing solutions were varied, an aqueous solution of the iron salt being used, and a glycerine solution of potassium ferrocyanide. By this method a blue colour was invariably seen on the free surface after a certain number of hours, the degree varying with the method of procedure. The microscopical examination of the tissue, fixed in absolute alcohol, furnished no evidence of blue colour in the cells, but a slight deposit of Prussian blue was occasionally observed in a rift running parallel to a gland, and perpendicular to the surface, regarded by Lepine as, in all probability, a lymphatic channel. He explained this as due to the lymphatic space readily permitting the osmosis of the two fluids, and as iron solutions are always slightly acid (a solution of sulphate and lactate of iron always giving an acid reaction), the conditions sufficed for the formation of Prussian blue.

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* R. Lepine, 'Comptes Rendus,' 1872, vol. 24, p. 221; and 'Gaz. Heb. de Méd.,' Paris, 1873, p. 691.

The gastric mucosæ of dogs, killed by section of the medulla during full digestion, were also placed in contact with a litmus solution, but in these cases an acid reaction was not obtained.

From these varied experiments Lepine concluded that the cells of the gastric glands did not possess an acid reaction, but he also stated that the failure to demonstrate acid did not prevent one kind of cell possessing the function of preparing the acid, if not of completely elaborating it. In this connection he refers to the work of Ebstein and Grutzner,* in which the view is expressed that probably the secretion elaborated by the parietal cells is rich in chlorides of the alkalies, this, by an unknown process, together with the inactive pepsin from the chief cells, becoming acid first on the free surface of the gastric mucosa.

In a discussion that followed the reading of the report of Lepine's earlier experiments (p. 60),† Vulpian did not regard the results as decisive, and Rabuteau recommended the substitution of sodium ferrocyanide for the potassium salt, because of the rapidly fatal action of the latter on cells, which possibly prevented their coloration. Ranvier, however, stated that by slow injection of small doses an enormous quantity of the potassium salt could be introduced into rabbits without causing their death. In his own experiments he observed that the diffusion of the salt was not the same in all the tissues and fluids, and thought that Lepine's negative results were due to this inequality of diffusion. He found the salt present in great quantity in the connective tissue and lymph, but not in the muscles. He also found that the elimination of the salt was very rapid, that it made its appearance very rapidly in the urine, and was present in great quantity, the urine at the same time being increased in amount.

In an abstract of Lepine's work, Maly‡ makes the criticism that the alkaline solution used was not free from objection, since it would contain the colloidal ferric hydrate, and therefore, owing to the lack of diffusion of one of the salts, Prussian blue could not be deposited within the cell if acid were present.

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* W. Ebstein and Grutzner, 'Pflüger's Archiv,' 1874, vol. 8, p. 150.

† 'Comptes Rendus,' 1872, vol. 24, p. 221.

‡ R. Maly, 'Jahresb. d. Thier. Chem.,' 1873, p. 174.

Various micro-chemical reactions were employed by Edinger in 1882.* Hoping to solve the question by only colouring alkaline tissue, this investigator injected into a rabbit a colourless solution of phenolphthalein, which became of a deep red colour in the presence of alkali, but as the results obtained were lacking in clearness this method was abandoned.

More promising but indefinite results were obtained by the use of a neutral concentrated solution of sodium alizarin. From this solution, purple-red in colour, is precipitated on the addition of a drop of acid the golden-yellow colloidal alizarin, insoluble in water. The precipitation was brought about by all acids, but of the salts investigated by him Edinger found that it was caused only by potassium and sodium phosphate. He considered this solution suitable for the enquiry on account of (1) its extreme sensitiveness to acid (0·0007 HCl, Lieberkühn); and (2) the colloidal nature of the precipitate.

He accordingly injected intravenously a solution of the above-named salt into rabbits and dogs, and as a result found the gastric mucosa flecked with a yellow colour. These orange-yellow areas corresponded to gland groups, and were separated one from another by broad red-violet zones. In some places the yellow areas were confluent. On making sections of the mucosa corresponding to one of these yellow areas, the colour could be traced into the deeper part of the tissue, in many cases throughout the length of the gland, in others through the upper third or to the middle of the gland tubule, but unfortunately in thin sections the colour was too faint for positive information of its precise situation to be obtained; therefore, knowledge of the function or reaction of the cells of the gastric glands was not furthered. He considered these results proved that the acid reaction of the gland tissue is spontaneous, but is not evident in all the glands at the same time, the red areas corresponding to resting glands. The reaction disappeared quickly or entirely in hunger (dogs). Owing to the rapid disappearance of the acid reaction from dying tissue, the animals were killed immediately after the injection if they had not already succumbed. The application of acid or alkali to the red or yellow spots caused an immediate alteration to yellow or red respectively, and this test could be repeated many times.

In the rabbit the golden islands, separated by red-violet zones, occurred in the fundus, once in the anterior region of the lesser curvature, and in the region of the greater curvature, where they were particularly large, owing to confluence. The pyloric region was of a dirty yellow or red-yellow colour. Edinger mentions that his results confirm those of Lieberkühn,* who also observed the yellow colour of the stomach following the use of alizarin.

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* L. Edinger, 'Pflüger's Archiv,' 1882, vol. 29.

Tropæolin was another reagent used by Edinger. This yellow dye, known as Tropæolin OO, is unaffected by organic acids, but is exceedingly sensitive to mineral acids, the smallest trace of the latter being sufficient to cause the colour to change to carmine red. It was brought into medical technique for determining the presence of free hydrochloric acid in the gastric juice by Von den Velden,† who used it in the form of a crystalline salt of potassium and not in the brown commercial state. He pointed out that in the presence of much peptone, or of a small quantity of albumen, the colour change does not occur on the addition of hydrochloric acid.

Edinger also points out that in the presence of strongly saline fluids the reaction does not occur. He applied the reagent by injecting a solution into the large artery of the stomach of fasting dogs killed just before injection. He found no trace of red colour in the gastric mucosa, and obtained a yellow colour everywhere. Previous to this, Edinger‡ had employed tropæolin in an investigation of the stomach of the frog with negative results.

Trinkler§ also employed tropæolin. After carefully washing the stomach of the dog, he placed the teased glands in a solution of the dye and made his observations under the microscope. He found both the parietal and the chief cells yellow, the tint of a neutral solution of tropæolin, and therefore concluded that neither kind of cell held or formed free acid. He was further strengthened in his view by the negative results obtained with litmus.

Stintzing|| endeavoured to determine the acid-forming element by means of Congo red, which changes in colour from deep red to blue in the presence of a trace of hydrochloric acid, and forms a firm compound in either alcoholic or aqueous solution. He applied the red solution to the gastric mucosa of the following animals:—Frog, mouse, rat, guinea-pig, and rabbit, and in each case within certain cells of the fundus glands he detected blue granules, sometimes small, sometimes the size of a cell nucleus. Although not venturing to affirm that these results proved the existence of acid within these cells, which could be classed as parietal cells, he held the opinion that they furnished some supporting evidence of the formation of acid in the parietal cells.

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* Lieberkühn, 'Sitzungsb. d. Gesell. z. Beförd. d. ges. Naturwiss. zu Marburg,' 1874. Original paper unobtainable for reference.—M. P. F. G.

† Von den Velden, 'Deutsch. Archiv Klin. Med.,' 1879, vol. 23; 1880, vol. 27.

‡ L. Edinger, 'Archiv Mikros. Anat.,' 1879, vol. 17.

§ N. Trinkler, 'Archiv Mikros. Anat.,' 1885, vol. 24.

|| Stintzing, 'Münchener Med. Wochenschr.,' 1889, p. 793.

The Prussian blue method was again resorted to by Sehrwald in 1889.* He criticised the experiment of Claude Bernard, and stated that in it the chemical premise was wrong, as the lactate of iron and potassium ferrocyanide had been employed for the production of Prussian blue in the presence of an acid; and such a result could not follow, since the iron in the lactate acts as a divalent metal, and therefore the salt belongs to the ferrous oxide compounds, which will not form Prussian blue with potassium ferrocyanide either in the presence or in the absence of an acid, a ferric salt being necessary for such a formation. He also says that if Claude Bernard obtained Prussian blue in the stomach, notwithstanding his chemical error, this would be due to the inherent tendency of ferrous salts to oxidise and therefore change to ferric salts, which with potassium ferrocyanide will form Prussian blue in an acid solution. Through this tendency, which would naturally persist in the body, the blue colour could appear in the acid parietal cells.

Sehrwald, therefore, used lactate of iron together with potassium ferricyanide, which, in an acid solution, forms Turnbull's blue (Prussian blue). To avoid the possibility of the reaction being prevented by one or other of the salts undergoing change in the body, he abandoned the injection method, and placed small sections of a fresh stomach, first, into a solution of lactate of iron for one day, and thence, after washing quickly, into a solution of potassium ferricyanide.

As Lepine before him, Sehrwald had observed the acid reaction of different iron salts in solution, including the lactate, and in such cases, without the further addition of an acid, that the formation of Prussian blue would occur when solutions of an iron salt and potassium ferricyanide were brought together. Taking this fact into consideration, he argued that if the cells were alkaline the acidity of the reagent would be neutralised in part and the cells would be uncoloured, and that if this alkalinity exceeded a certain degree the cells must remain so, but if acid, they would become intensely blue in colour.

As the result of his experiment, he found that the parietal cells had become deep blue in colour, while the chief cells were absolutely colourless. He therefore concluded that the parietal cells were less alkaline than the chief cells—the alkaline reaction being demonstrated by their colourless condition—and that the intensity of the blue colour in the parietal cells pointed to their being neutral if not acid in reaction, and strongly supported the view of their forming the acid.

Sehrwald also observed a blue colour on the surface of the mucosa, and in the outer border of the chief cells in the necks of the glands; this he interpreted as due to early *post-mortem* changes permitting the imbibition of acid from the stomach contents. Dark blue masses were also found filling the lumina of the neck region of the glands in certain places; these he regarded as acid ingredients of the stomach and masses of mucus. By the same method Sehrwald found that the connective tissue and the walls of the blood-vessels became blue, and very frequently the blood corpuscles also, enabling even small capillaries to be traced. The production of colour in such corpuscles he regarded as due to the small quantity of plasma or lymph in their immediate neighbourhood being insufficient to neutralise the acid of the iron salt, and thus, when taken up by the cells, coloration would be rendered possible.

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* Sehrwald, 'Münchener Med. Wochenschr.,' 1889, p. 177.

This occurrence might also be explained by the supposition that, as regards the acid of the iron salt, the absorptive power of certain cellular elements—the connective tissue elements, the parietal cells, and certain of the red corpuscles included—is so great that, although they were previously alkaline in reaction, as a result of this absorption they take on an acid character. This, together with the fact of the experiment being based on the assumption that the acid reaction of the parietal cells would persist throughout the day the sections were allowed to remain in the solution of lactate of iron before being placed in one of potassium ferricyanide, and that the acid fluid, if present, would not diffuse from, or the alkaline fluids of surrounding elements would not diffuse into the parietal cells and neutralise any acid present, makes it evident that the results obtained cannot be regarded as decisive.

A criticism similar to the above was passed by Fränkel in 1891,* and he further states that if sections of a stomach, hardened for some time in alcohol, are placed in a solution of Prussian blue, the same effect is produced that Sehrwald regarded as evidence of the formation of acid by the parietal cells.

Sehrwald† also made experiments *in vitro* with anilin black, for the purpose of deciding whether the differential staining of the parietal cells, observed by Grützner when using this dye, was due to their acid reaction. The latter observer‡ had found by treating sections of the stomach fixed in alcohol with 1-per-cent. aqueous solution of anilin black, and subsequently with bichromate of potassium, that the parietal cells became violet-black in colour, while the chief cells were of a dirty grey hue with blackish nuclei. He did not interpret this as due to an acid reaction of the parietal cells, but Heidenhain had previously suggested this as possibly responsible for such cells being stained by anilin blue.*

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* S. Fränkel, 'Pflüger's Archiv,' 1891, vol. 48.

† Sehrwald, *ibid.*

‡ Grützner, 'Pflüger's Archiv,' 1879, vol. 20.

Sehrwald found that if hydrochloric acid was added to a dilute solution of anilin black, which is of a blue colour in transmitted light, the blue was unchanged, but that, on the addition of alkali, the colour changed to a dirty pale violet, with no resemblance to that of the original solution. On the addition of potassium bichromate, the blue and pale violet colour disappeared respectively from the acid and alkaline solutions, but a striking difference was observable in the intensity and character of the colour then attained, the acid solution being grey blue, and the alkaline pale grey; with highly concentrated solutions, the colour difference obtained was similar to that shown by Grützner to prevail in the parietal and chief cells of preparations of the gastric mucosa stained by anilin black. Sehrwald therefore concluded that the darkly-stained parietal cells, described by Grützner, were richer in acid, and the pale grey chief cells more alkaline, and that these results furnish evidence of acid formation in the parietal cells.

Fränkel† repeated the alizarin experiment of Edinger on the dog, and fully confirmed the results obtained by the earlier investigator, but could not determine in which cellular element the acid occurred. He also points out that an objection can be raised to the use of sodium alizarin for the end in view, since the reaction may be brought about by acid resulting from dissociation of the neutral salt.

Fränkel therefore turned his attention to another reagent. Following the method employed by Dreser‡ to demonstrate the presence of acid in muscle during activity, he injected into the jugular vein of a dog a 5-per-cent. solution of acid fuchsin, previously decolourised with either caustic soda or with carbonate of soda, the latter being usually employed. The amount injected varied from 50 to 100 c.cms., according to the size of the animal. Prior to injection, the gastric juice of the dog to be experimented upon was tested with phloroglucin and vanillin and with Congo paper. The dogs used were in full digestion. As result, the mucosa, macroscopically, was seen to be of a beautiful red colour, the pylorus region included, while the œsophagus and duodenum were uncoloured. From the microscopical examination of teased portions of the mucosa placed in a drop of distilled water, he found both the parietal and chief cells, readily distinguishable one from another, intensely red, the colour of both kinds being of equal intensity.

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* Heidenhain, 'Handbuch d. Physiol., Hermann,' 1883, vol. 5, p. 150.

† S. Fränkel, *ibid.*

‡ H. Dreser, 'Centralb. f. Physiol.,' 1887, vol. I.

The cylindrical epithelium and the interstitial tissue were, on the other hand, uncoloured.

In repeating the experiment with the rabbit, the results obtained were, in the main, the same; but in this animal, owing to the glands not being uniformly active, the mucosa was flecked with red instead of being uniformly coloured, while the intervening tissue was of the normal hue. The pyloric region was also uncoloured, except in one instance, when faintly acid spots were observed. In the microscopical examination of teased glands taken from uncoloured areas, no red colour whatever was seen, nor was the red colour peculiar to one kind of cell in the borderland between a coloured and an uncoloured area.

In testing the reagent used, Fränkel found that all acids would produce the red colour, also acid phosphates, and acid ammonium tartrate, but that neutral salts would not. He regarded the results obtained as proving the acid reaction of the gastric mucosa and the formation of the acid in the gland cells, but did not feel justified in saying that this formation could be ascribed to one or other kind of cell exclusively. He considered the view held by others that only one kind of cell produced the free acid, was by no means established.

Oppel* and Gmelin† also hold the opinion that an inter-relation between the parietal cells and hydrochloric acid is not yet proven. Gmelin, in the course of the experiments upon which he based this opinion, stained the parietal cells of the puppy with Congo red and obtained a brown colour.

During the course of the years in which the experiments previously mentioned were performed, indirect evidence in support of Heidenhain's theory of the acid-forming function of the parietal cells was supplied from time to time by experimental work of another kind, a complete account of which is outside the scope of the present paper.

Of special interest in reference to the hydrochloric acid originating from the chlorides of the organism are the observations of Mary Greenwood made in 1884–5.‡ She found, when treating the gastric glands of the pig with silver nitrate, that the parietal cells stained readily and deeply with the reagent on exposure to light, the reducing portion of the cell apparently being the interstitial substance and not the network of the cytoplasm.

She further found that the cells of the gastric glands of the frog, which secrete an acid fluid, gave a colour reaction with the silver compound, while those of the œsophagus, which secrete an alkaline fluid, did not. She therefore regarded the reaction as indicating the presence of chlorides in the parietal cells, and associated this with their acid-forming function.

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* Oppel, 'Lehrbuch der vergleich. Mikrosk. Anat. I. Theil,' "Der Magen," 1896, p. 253.

† Gmelin, 'Archiv für d. ges. Physiol.,' 1902, vol. 90, p. 103.

‡ M. Greenwood, 'Journ. Physiol.,' 1884–1885, vol. 5, p. 195.

Though giving support to the presence of chlorides within the parietal cells, the results cannot be regarded as proving the point, for Macallum* has shown that besides the chlorides other silver salts, such as the phosphate, carbonate, etc., produce a coloured reduction compound under the action of light, and that it is only in the presence of free nitric acid, which prevents the formation of these other coloured reduction compounds, that the reaction of silver nitrate can be relied upon for the detection of chlorides in animal and vegetable tissues, and in this case also taurine and creatine must be absent, since they give a reaction similar to that of the chloride in an acid solution.

Before accepting the conclusion of Mary Greenwood, or attributing the reaction to the presence of hydrochloric acid in the cells, it would, therefore, be a matter of especial importance to eliminate the possibility of the reaction being due either to the presence of phosphate of sodium, the diacid form of which is supposed by Maly† to be the effective agent in bringing about the production of hydrochloric acid from sodium chloride, or to other organic compounds of physiological origin, which will also in the absence of free nitric acid produce a dark reduction compound with silver nitrate under the influence of light.

This has been accomplished by Macallum,‡ who at the same time proved by the use of his silver nitrate—nitric acid method that in comparison with the chief cells and adjacent tissues chlorides were present in abundance in the parietal cells of the gastric glands of the rabbit and guinea-pig, the reaction being so pronounced as to readily demonstrate the occurrence of these cells along the course of the tubules.

From this necessarily condensed account of even a portion of the work done it will be seen that hitherto certainty has not been attained in reference to the structure elaborating the hydrochloric acid of the gastric secretion, although the view that its elaboration was in some way connected with the parietal cells has been maintained. Neither has it hitherto been proved that the hydrochloric acid exists in a demonstrable form in the secretion of the glands before this reaches the free surface of the mucosa. Therefore, in spite of all endeavour during the intervening years, the outlook of Bickel in 1908 (see p. 56) closely resembles that of Prout in 1823.

Notwithstanding so many failures, the opinion was held by Prof. A. B. Macallum that micro-chemistry would still reveal the seat of formation of the hydrochloric acid, and by some modification of technique or change of salt the Prussian blue reaction in particular might still be of service in the solution of the problem.

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* A. B. Macallum, 'Roy. Soc. Proc.,' 1905, B, vol. 76.

† R. Maly, 'Zeitsch. f. Phys. Chem.,' 1878 vol. 1, p. 174.

‡ A. B. Macallum, 'Ergebn. der Physiol ' 1908, vol. 7, p. 628.

This opinion has received its justification, and results of a positive nature have been obtained by the substitution of the double citrate of iron and ammonia for the lactate of iron as used by Bernard in conjunction with potassium ferrocyanide.

For the purpose to be accomplished, and the methods to be employed, the double citrate of iron and ammonia was found to be a salt free from objectionable qualities inherent in the lactate and other salts of iron previously employed in Claude Bernard's method. In its officinal preparation, and to meet the requirements of the pharmacopœia, the salt is generally made by dissolving freshly precipitated ferric hydrate in citric acid. Ammonia is added until the neutral point is reached, and the solution is then evaporated to the consistency of a syrup. In this condition it is spread out in thin layers on porcelain plates, and completely dried. The resulting scale-like residue is not uniform in its composition, but as a rule does not contain less than 18 per cent. of iron, and may contain considerably more. The salt gives either a very feeble reaction to blue litmus paper or no reaction at all. The reaction of the sample used in the experiments was very faintly alkaline to red litmus paper; and in aqueous solution the salt possessed a sweetish taste and very faintly astringent properties.

If an aqueous solution of the double citrate is added to one of potassium ferrocyanide a formation of Prussian blue does not occur, nor will it have developed at the end of 24 hours. But if hydrochloric acid is added to the mixture, sufficient in quantity to make it of 0·03645 per cent. strength, which is a concentration less than that existing in the gastric juice, the Prussian blue reaction begins to develop and becomes more marked at the end of two hours, the colour of the mixture at the outset being greenish. If the acid added corresponds to 0·0729 per cent. strength, the formation of Prussian blue begins very quickly, and if it is of greater strength, *i.e.* 0·1 per cent., the reaction is both immediate and intense.

The addition of sodium dihydrogen phosphate, or of disodium hydrogen phosphate, however concentrated, to a mixture of the double citrate and potassium ferrocyanide in solution will not produce Prussian blue, even during the course of 24 hours. Neither will carbon dioxide produce the reaction, even when present in such a mixture under considerable tension.*

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* The addition of the corresponding phosphates of potassium, or of mono-sodium carbonate, also gives a negative result. A series of experiments showed further that a Prussian blue reaction developed only after the ammonium ferric citrate and potassium

Prussian blue is not formed by the addition of a solution of the double citrate to one of potassium ferricyanide, even when acid is present, showing that the iron of the compound is all in the ferric state.

It is thus seen that the double citrate presents none of the disadvantages pertaining to the use of other iron salts for subcutaneous or intravascular injection, either alone or in conjunction with a solution of ferrocyanide of potassium, and, moreover, when it gives in solution with the latter a distinct Prussian blue reaction, the additional presence of an acid, *i.e.* of extraneous origin, can be regarded as certain, whereas with other iron salts such a certainty cannot exist, as an acid may arise from the dissociation of such salts. For example, in a solution of ferric chloride, hydrochloric acid, arising from the dissociation of the salt, is present in sufficient quantity to cause an immediate formation of Prussian blue on the addition of potassium ferrocyanide to the solution. Owing to the acid thus inherent, a similar formation occurs with the acetate, lactate, phosphate, sulphate (ferrous and ferric), nitrate, and iodide of iron, as well as with the ferrous and ferric ammonium sulphates, and, consequently, all such compounds are more or less unsuitable for the purpose and methods of an investigation such as the present.

The preparation of the double citrate used in these experiments was found by analysis to contain 25·7 per cent. of iron. Therefore, to ensure a solution of it and of the potassium ferrocyanide being so balanced that, on addition of a sufficient quantity of acid, all the iron of the citrate and all the $Fe(CN)_6$ atom groups would go out of solution as Prussian blue, it would be necessary to have present in solution five parts of the ferrocyanide to approximately every three parts by weight of the double citrate dissolved.

In this investigation, however, the solution used contained 1·5 per cent. of potassium ferrocyanide and 2·25 per cent. of ammonium ferric citrate, proportions which did not give a balanced solution as designated above. For the purpose of intravenous or subcutaneous injection, such balanced solutions are better than unbalanced ones, only, if the rate of diffusion of the two salts is the same through membranes, vascular walls, and cellular structures, which has not been ascertained to be the case.

ferrocyanide solutions had been standing in contact for from 36—48 hours with the following :—

(1) Potassium dihydrogen phosphate.
(2) Potassium dihydrogen phosphate and carbon dioxide.
(3) Carbon dioxide.
(4) Potassium dihydrogen phosphate and mono-sodium carbonate.

The reaction was very slight in the tubes containing the latter compounds.

It is likewise not known that either salt diffuses through all the tissues of the body with a uniform velocity, the observations of Ranvier previously mentioned, p. 61, point to the contrary in the case of potassium ferrocyanide, so that a solution which in a test-tube is "balanced" may not be so in the gastric mucosa. Therefore it is better for the object in view to have an excess of the double citrate, so that in the gastric tubules a complete disposal of all the ferrocyanide can be effected, and thus enable the gland-cells engaged in the formation of hydrochloric acid to demonstrate the presence of the acid in such structures.

The animals selected for the investigation were the dog, the rabbit, and the guinea-pig. Their physiological condition as regards digestive activity varied from one of semi-starvation to one of full digestion.

The injection fluid was freshly prepared at the time of injection by taking equal parts of a 1·5-per-cent. aqueous solution of potassium ferrocyanide and of a 2·25-per-cent. aqueous solution of ammonium ferric citrate.

In one instance sodium ferrocyanide was substituted for the potassium salt, but no appreciable difference in results was observed.

The total amount of fluid injected, by two or more injections given at varying intervals, varied from 10–11 to 45 c. cms. in rabbits; from 16 to 22 c. cms. in guinea-pigs; and from 42 to 55 c. cms. in dogs.

The subcutaneous method was employed as a rule, and was found to give the best results. The flank was the region selected for injection in the dog and the rabbit, and the abdominal region in the guinea-pig. In one case (rabbit) the injections were given intravenously (lateral branch of the posterior auricular vein), and in another (rabbit) an intravenous injection was supplemented by two given by the subcutaneous method.

In many instances, but not in all, toxic effects were produced during life by the solution injected, and these varied somewhat in the different animals. By a process of exclusion, the toxic effect could be attributed to the potassium ferrocyanide, as experiments made by Prof. A. B. Macallum showed that large doses of the double citrate could be injected into rabbits without causing any physiological disturbance.

The toxic effects were manifested by refusal of food, by discomfort, restlessness and agitation, or by lethargy. Inflammatory conditions supervened at the site of inoculation in the guinea-pig, and much gas was found in the stomach and intestines. Diuresis was observed in the guinea-pig, and there were indications of this also in the rabbit, but in the dog, diminution in quantity, or suppression, of urine ensued. In the first dog experimented upon vomiting occurred once, but apart from this no marked disturbance was observed. In the second dog lethargy was a pronounced symptom, and within three hours from the beginning of the experiment this had approached the comatose condition. The amount of the solution (ammonium ferric citrate and potassium ferrocyanide) injected in this case was 42 c. cms., two injections being given, with an interval of two hours between the first and the second. The weight of the dog was 12 lbs.

A general toxic effect was usually observed in rabbits which had received a large amount of the injection fluid during a short space of time, *i.e.* 40 to 50 c. cms. within the space of seven hours. Large amounts given over a long period were less likely to produce disturbance, and toxic effects were absent when small doses were given in a short space of time, *i.e.* 10 to 17 c. cms. in five hours.

These observations are in close agreement with some of those of Combemale and Dubiquet.* These investigators administered by the stomach an aqueous solution of potassium ferrocyanide to dogs and guinea-pigs, the doses varying from 0·08 gramme to 2 grammes per kilo. of body weight. They found that in the dog the repeated use of the salt caused an irritation of the alimentary canal, manifested by diarrhœa and vomiting, the latter symptom being invariably present when the dose attained or exceeded 80 centigrammes per kilo. of body weight, whatever the concentration of the fluid, and that suppression or diminution in quantity of urine sometimes ensued, but diuresis never, while in the guinea-pig diuresis was produced even by small doses, and the digestive tract was, as a rule, unaffected. They found further that the temperature, the circulatory, respiratory, and the nervous systems were not affected in any constant manner either in the dog or the guinea-pig.

These observers did not regard potassium ferrocyanide as toxic. They were also of the opinion that the ferrocyanide was transformed in the body to ferricyanide of potassium, and that in the guinea-pig it was eliminated in the urine entirely in this changed form, provided the dose did not exceed 45 centigrammes per kilo. of body weight, but that with a greater amount the power to transform the salt was lost, and ferrocyanide was then present in the urine. They held that the condensation of the ferrocyanide molecule took place probably in the blood, and, since ferricyanide of potassium did not cause diuresis in guinea-pigs, that the occurrence of this was due to the two atoms of potassium thus set free, forming salts of known diuretic action. They found the ferricyanide present in the urine as early as 45 minutes after the ingestion of the salt and in the third micturition, and in another case the urine gave the ferricyanide reaction as late as 20 hours and in the eighth micturition after ingestion of the ferrocyanide of potassium.

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* Combemale et Dubiquet, 'Compt. Rend. Soc. Biol.,' 1890, vol. 42.

A change, such as this recorded by Combemale and Dubiquet, in the potassium salt would not prohibit the occurrence of the Prussian blue reaction with certain of the iron salts, but would be absolutely prohibitive to any such formation with ammonium ferric citrate, for if a solution of this salt is mixed with one of potassium ferricyanide, the colour of the fluid remains brown, and becomes olive brown on the addition of hydrochloric acid.

On the addition of hydrochloric acid to the urine of the animals personally investigated (dogs, rabbits, and guinea-pigs), the Prussian blue reaction occurred in almost every case, thus proving the presence of unchanged potassium ferrocyanide, as well as that of the iron salt.

In cases of exception to this rule, proof of both the salts having existed in the body in an unchanged condition was furnished either by the presence of Prussian blue on the surface of the gastric mucosa, or by the occurrence of the reaction in one or other of the tissues treated after removal from the body with dilute hydrochloric acid, and tests were not made to ascertain which salt was lacking in the urine. The absolute exception, that is, entire disability to obtain the Prussian blue reaction in the urine, occurred in a dog and in a rabbit in which suppression and retention of urine were respectively observed. In the case of the dog (see Table, Dog 1), a very small quantity of urine was voided shortly before death, 3¼ hours after the beginning of the experiment, and the bladder was found to be empty at the *post-mortem* examination, while in the case of the rabbit (Rabbit 3) no urine, as far as known, was voided during the 25¾ hours of the experiment and the bladder was found enormously distended, but the bladder tissue in this case became slightly blue in the hydrochloric acid alcohol fixing solution. For the amount of solution injected see Table, Dog 1 and Rabbit 3. In the case of Rabbit 2, the Prussian blue reaction was obtained during the first day of the experiment, after 17 c. cms. of the injection fluid had been administered; but it could not be obtained in the urine of the bladder at the time of death, 30 hours after the first and 2½ hours after the last injection, when the total quantity of fluid injected had amounted to 37 c. cms.

The earliest time that the Prussian blue reaction was obtained in the voided urine was 1½ hours after the beginning of the experiment (Rabbit 6). A few minutes previous to this micturition, the rabbit had received the third injection of 11 c. cms., two others of the same amount having been given during the first hour of the experiment. The reaction at this time was faint, but was more marked in the urine voided three-quarters of an hour later.

The reaction was generally obtained in the urine, either voided during life or taken from the bladder after death, about 3 hours after the beginning of the experiment in each kind of animal.

Taking Rabbit 7, in which case sodium ferrocyanide had been substituted for the potassium salt, the amount of the injection fluid given prior to the obtaining of the Prussian blue reaction in the voided urine was 30 c. cms. The weight of the animal being 1·93 kilo. (4¼ lbs.), this would prove the presence of unchanged sodium ferrocyanide in a dose representing 23·3 centigrammes of sodium ferrocyanide per kilo. of body weight. The reaction was also obtained in the urine of the bladder after death, the total amount of the injection fluid received being 40 c. cms., or a dose equal to 31 centigrammes per kilo. of body weight.

Dog 2, whose weight was 5·45 kilos. (12 lbs.), received 42 c. cms. of the potassium ferrocyanide and ammonium ferric citrate solution; the Prussian blue reaction was obtained in the urine of the bladder at death 3¼ hours from the beginning of the experiment, so that evidence of untransformed potassium ferrocyanide was obtained in this animal after a dose equivalent to 11·5 centigrammes per kilo. of body weight.

Dating from the time of the first injection, the experiments varied in duration from 3 to 30 hours, and the animals were killed at times varying from three quarters of an hour to two hours after receiving the last injection. Death was caused in dogs by pithing, in rabbits by an anæsthetic (chloroform; in one case ether preceded chloroform), and in guinea-pigs by shock (blow over medulla). In every case, the stomach was removed and opened at the earliest possible moment after death, the mucosa exposed, and the surface of this, and of the contents, examined for a deposit of Prussian blue. The organ was then freed from the contents and placed in absolute alcohol for fixation. When sufficiently hard, thin vertical sections were cut by hand.

With the exception of the spontaneous occurrence of the Prussian blue reaction in the skin at the site of inoculation in one or two instances, the tissue of the stomach alone exhibited a blue colour at the *post-mortem* examinations. Evidence of the presence of Prussian blue on the surface of the gastric mucosa was obtained in each kind of animal; and, in the rabbit, Prussian blue was also demonstrated in the lumina of the tubules, and in the canaliculi branching off from these into the parietal cells.

A more complete examination of the stomach contents was made after their removal from the organ; extracts were made of these in distilled water, also of the contents of different regions of the intestines in the majority of cases, and the filtered fluid tested for the presence of iron salts and potassium ferrocyanide. In certain cases the bile was examined, but always with negative results. An arbitrary division of the contents of the stomach was made, and a portion taken from each of these, viz., from the contents of (1) fundus proper, (2) the intermediate region corresponding to the region of the œsophageal opening and the lesser curvature immediately adjacent, and (3) the pyloric region.

The production of the Prussian blue reaction was used as the general test, and the presence or absence of any one of the three necessary factors determined by adding, to a portion of the filtrate, one or both of the others as required. In many cases, in addition to this, hæmatoxylin was employed as a test for iron. In the application of the test, a minute quantity of a 1-per-cent. aqueous solution of the reagent was added to portions of the filtrates previously rendered neutral to litmus paper. As shown by Macallum,* dilute solutions of hæmatoxylin in pure water give a blue-black reaction in the presence of "unmasked" iron, but not in the presence of "masked" iron, under which category the ferrocyanide and ferricyanide fall.

The presence of iron was not demonstrated by the hæmatoxylin method in the filtrates of the gastric contents of the dog, nor could the Prussian blue reaction be obtained in these.

In those filtrates pertaining to the rabbit and guinea-pig in which it was found possible to produce a Prussian blue reaction, it was generally observed that a sufficiency of iron salt already existed, but that potassium ferrocyanide had to be supplied or augmented, and, in many cases, the hydrochloric acid also.

In the guinea-pig, the reactions were obtained in one instance only (Guinea-pig 3); in this it was found that, although sufficient acid existed for the production of the Prussian blue reaction in the filtrates of the different portions of the contents removed in the first place—the filtrate of the contents of the intermediate region being the most acid—both salts had to be added; and it was found necessary to make a filtrate of the "total" remainder of the contents to obtain sufficient iron for the spontaneous occurrence of the reaction on the addition of potassium ferrocyanide, as well as for the demonstration of iron by the hæmatoxylin method. In Guinea-pig 2, sufficient acid was present in the filtrate of the fundus and of the "total" contents, but the salts were lacking; both the acid and the salts were lacking in the filtrates of the contents of the intermediate and pyloric regions.

In the rabbit, the Prussian blue reaction was rarely obtainable in the filtrate of the contents of the fundus region proper, but in the case of Rabbit 7, in which the solution injected had contained sodium ferrocyanide instead of the potassium salt, the reaction was produced in the filtrate of the contents of this region to a marked degree. A similar result was obtained in Rabbit 3. On the other hand, the reaction was usually obtained in the filtrate of the contents removed from the intermediate region; or, in other words, in the filtrate of the contents contiguous or adjacent to that part of the mucosa to which the Prussian blue deposit generally adhered. Occasionally, a very faint reaction was obtained in the filtrate of the contents of the pyloric region. In Rabbit 6, the hæmatoxylin reaction was obtained in the filtrate pertaining to the intermediate region, and in that of the upper part of the pyloric region; but not in the filtrate of the contents removed from the superior half of the organ in this region. It is of interest to note that in the control (uninjected) rabbit, the Prussian blue reaction was also obtained in the filtrate of the contents of both the intermediate and pyloric regions, on the addition of hydrochloric acid and potassium ferrocyanide; but the reaction was slight in comparison to that obtained in the injected animal. This appears to indicate an excretion of iron by the cells of the gastric mucosa, since only a small quantity of iron could have been derived from the food (carrots). In the analysis of carrots given by Röttger,* the ash represents 1·03 per cent. of the total material, and of this ash, iron (Fe_2O_3) represents 1·01 per cent. In certain instances, notably in Rabbit 3, the Prussian blue reaction was obtained in the filtrate of the contents of the upper portion of the duodenum.

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* A. B. Macallum, 'Journ. Physiol.,' 1897, vol. 22, p. 92.

As before stated, a deposit of Prussian blue was observed adhering to the surface of the mucosa and in the gastric contents of each species of animal experimented upon, being present, frequently to a marked degree, in the majority of the rabbits, in each of the three guinea-pigs, and, in a minor degree, in one of two dogs employed.

The earliest observed occurrence of the deposit on the surface of the mucosa was three hours after the first injection; but as a minimum period of three hours, dating from the first injection, was allowed to elapse before any of the animals were killed, no information can be supplied from the present series of experiments as to the earliest appearance of the deposit. It will, however, be recalled that Claude Bernard observed a superficial deposit three-quarters of an hour after the injection of the lactate of iron and potassium ferrocyanide.

A statement in reference to the earliest observed excretion by the kidneys of the two salts injected in solution will be found on p. 73.

In the rabbit and the guinea-pig the Prussian blue thus observed on opening the stomach was invariably situated in a comparatively speaking circumscribed area in the œsophageal region, close to the opening of the œsophagus, and in the region of the lesser curvature adjacent to this.* In the guinea-pig the deposit of Prussian blue was found to occur further to the left of the œsophagus, *i.e.* further towards the fundus, than in the rabbit; but in neither of these animals was it detected in the fundus region proper, nor in the superior half of the organ.

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* Röttger, 'Nährungsmittel-Chemie,' 3rd ed., p. 344.

In the dog the deposit was also found in the inferior half of the organ, and in a region slightly to the left of the œsophagus in the direction of the fundus, but further evidence would be required before definite statement could be made as to the usual situation of the deposit in this animal.

A case of special interest, in which there was an extension of the area wherein the Prussian blue deposit usually occurred, was that of a rabbit (Rabbit 5, see Table) which had received a large amount of the ammonium ferric citrate and potassium ferrocyanide solution in a series of five injections during a comparatively short space of time (50 c. cms. within 6½ hours, injected in quantities of 10 to 11 c. cms.). The animal was killed 6½ hours after the last injection. The injection solution had exerted a toxic influence, manifested by quietude and refusal of food (carrots) offered during the experiment.

The Prussian blue reaction occurred with such intensity that on the removal of the stomach from the body the deposit on the mucosa was visible through the stomach wall, appearing as a diffuse green coloration from the outer surface. A diagram (fig. 1) from a drawing made at the autopsy is given to show the area over which the colour was visible through the wall of the unopened stomach.

Indications of colour similarly transmitted through the same region of the stomach wall were obtained in other instances in a minor degree.

On opening the stomach the mucosa presented a remarkable appearance (see Plate 7, fig. 1), for in the region indicated a total area of approximately 11 or 12 sq. cms. was covered with a thick deposit of Prussian blue of great intensity of colour. The deposit was greatest in amount in the region of the œsophageal opening and in the adjacent region of the lesser curvature. The sharpness of the demarcation of the deposit in a line with the base of the œsophagus was a noteworthy feature. In a vertical direction the deposit covered about one-half of the stomach wall. In addition to the deposit in

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* The portions of the gastric mucosa designated in this communication as the œsophageal region and the adjacent region of the lesser curvature correspond to the inferior half of the median part of the greater curvature and to a portion of the inferior half of the smaller curvature in the arbitrary divisions of the rabbit's stomach given by Langley. (J. N. Langley and H. Sewall, 'Journ. Physiol.,' 1879–1880, vol. 2.)

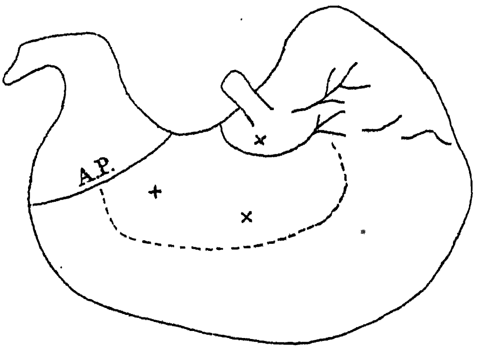

Fig. 1.—Diagram of Unopened Stomach of Rabbit. (Rabbit 5.) ⅜ size, ventral aspect. Crosses (×) are placed in region in which occurred the diffuse green coloration transmitted from the deposit of Prussian blue within stomach.

Broken line indicates outer limit of visible colour; A.P., Antrum pylori.

the usual situation in the region before named, a further deposit, slightly separated from the main deposit, covering an area of about 2 sq. cms., was found in the pyloric region beneath the antrum, extending to within 6 mms. of the pyloric opening. This was the solitary instance of an occurrence of a deposit of Prussian blue in the pyloric region; whether due to the intensity of the reaction and mechanical transference, or to a cause inherent in the mucosa of this region, can only be ascertained by further experiments. In another rabbit, in which the mucosa was tested with litmus paper, an acid reaction was obtained in the pyloric region, although less marked than in the other regions of the stomach.

Ebstein* found that the surface of the gastric mucosa of the rabbit in the pyloric region was acid in reaction, and it will be remembered that this was also observed by Edinger† when experimenting with sodium alizarin (see p. 62), and by Fränkel also, on one occasion, when using a decolorised solution of acid fuchsin (see p. 67).‡

A diagram is given of the dorsal wall of the opened stomach of the rabbit (Rabbit 5) to illustrate the region in which the Prussian blue was usually found, its maximum observed extent, as well as the additional and unusual area in the pyloric region. It is copied from a drawing of the stomach in which the areas covered by the deposit were carefully depicted, immediately after the mucosa was exposed.

The contents of the stomach (Rabbit 5) contiguous to the areas of the mucosa over which the Prussian blue was deposited were impregnated with

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* 'Archiv Mikros. Anat.,' 1870, vol. 6.

† Edinger, *ibid.*

‡ Fränkel, *ibid.*

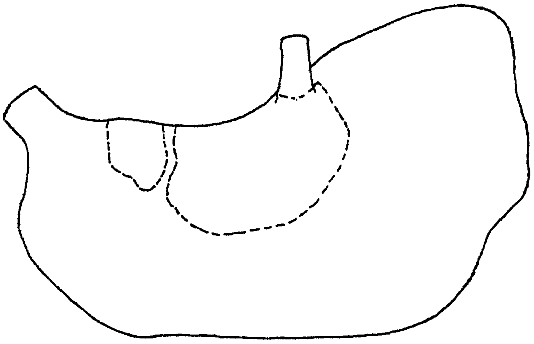

FIG. 2.—Diagram of Dorsal Wall of Opened Stomach of Rabbit (Rabbit 5), illustrating region in which Prussian blue deposit usually occurred, maximum intensity of occurrence of reaction, and unusual area covered by deposit in pyloric region. ⅗ size.

the blue compound, and this coloration extended for a considerable depth beneath the contiguous layer in the region of the œsophageal opening and of the lesser curvature adjacent to it. On the other hand, the contents of the fundus and of the superior half of the organ, as well as the greater portion of the contents of the pyloric region, were of the yellow colour normally found with a carrot diet, and exhibited no trace of the presence of Prussian blue.

On testing the filtrates obtained by extracting portions of the contents removed from (1) the fundus region; (2) the region of the œsophageal opening and of the lesser curvature adjacent to it; and (3) the pyloric region, proof was not obtained of the presence of either the iron salt or potassium ferrocyanide in the filtrate of the fundus contents, but the Prussian blue reaction occurred to a marked degree in the filtrate of region (2), and to a slight degree in that of region (3), on the addition of a small quantity of 0·5 per cent. hydrochloric acid and of one drop of a 1·5-per-cent. solution of potassium ferrocyanide, bearing witness to the presence of an iron salt, and, therefore, to the salt in question, or possibly to more than one of the factors necessary to the reaction, being mixed more particularly with the contents of region (2).

The contents of the intestines, similarly extracted, and the bile were likewise tested, but with negative results.

On the addition of 0·5 per cent. hydrochloric acid the Prussian blue reaction was obtained in the urine voided four hours after the first injection. Further interesting points observed in the same rabbit (5) will be dealt with later.

In some instances the examination of sections of the tissue underlying the superficial deposit of Prussian blue furnished no evidence of the presence of the blue compound. In others, it was observed only in the upper portion of the crypts of the glands; on the surface of the gland cells, or in structures external to the gland tubules; but in one of the rabbits proof of the most decisive character was obtained, both of the presence of the deposit of Prussian blue in the central lumina of the gland tubules and of the occurrence of the reaction within the parietal cells.

This rabbit (Rabbit 2, see Table) received eight injections by the subcutaneous method, during the space of 30 hours. The total amount of the solution of the two salts injected was 32 c. cms., and this was given in quantities varying from 4 to 6 c. cms.; 23 c. cms. were given during the first 12 hours of the experiment, the five injections being given at intervals of 1½ to 1¾ hours. After receiving this amount (23 c. cms.) the rabbit was allowed to rest for about 17 hours. During the subsequent seven hours three injections of 4 to 5 c. cms. of the fluid were given at intervals of 2 or 2½ hours.

The animal partook heartily of carrots supplied during the experiment. No toxic effect due to the injection fluid was observed. The earliest evidence of the excretion of the two salts contained in the injection fluid was obtained in the urine voided about six hours after the first injection. The rabbit was killed 30 hours after the first and 2½ hours after the last injection had been administered.

In this instance, only a slight deposit of Prussian blue was found adhering to the surface of the gastric mucosa and in the contents directly in contact with it, and this deposit, greenish in colour, was situated in a small area close to the œsophageal opening, and in the region adjacent, corresponding to the border of the lesser curvature.

In addition to the superficial deposit, the examination of vertical sections, with the low power of the microscope, revealed a deposit of Prussian blue in some of the crypts, and at isolated points in the upper two-thirds of the central lumina of certain of the gland tubules. (See Plate 7, fig. 2.) Occasionally the deposit extended continuously through more than a third of the gland to the surface, but more frequently it occurred in isolated lengths, and was as a rule confined to that third of the tubule adjoining the crypt. The deposit was never found in the lower third of the tubule. It is thus seen that the reaction occurred in the neck of the gland, in which region the parietal cells predominate. By further examination with the high power, the crypts appeared to be lined and filled with a material composed of wavy blue strands, frequently of considerable thickness. In these, deeply coloured varicosities occurred at irregular intervals, which were regarded as being probably agglomerations of the Prussian blue and mucus.

At the points mentioned of the tubules in which the blue deposit was observed, the central lumina was found to be thickly lined with aggregations of blue granules of irregular size and shape, causing the lumina to present a wavy or jagged appearance. (See Plate 7, figs. 3, 4.)

Besides the occurrence of the deposit already observed with the low power, lines of blue in certain instances could be observed passing between the central lumina and the parietal cells, and ramifying within these. As a rule only one, but occasionally two, of these side branches penetrated a cell. (See Plate 7, fig. 5.) Sometimes as many as three or four branches connected at short intervals from each other with the central lumen containing Prussian blue, were seen to pass towards an individual cell. In some cases a branch passed partly round a cell before entering it; in others, a branch curved round a cell and disappeared as if to reach a parietal cell situated at a greater distance from the lumen or in another plane, while another branch in close proximity entered the cell in the immediate neighbourhood. Occasionally branches extending to opposite sides were connected with the central lumen at the same point. On entering the cell the branch usually divided and subdivided, the fine canaliculi penetrating the depth of the cell. Local aggregations of the Prussian blue in the delicate canaliculi gave them, in many instances, a beaded appearance. Their course was frequently very wavy or serpentine.

Branchlets were frequently directed towards the nucleus; in many cases they curved round it to reach the cytoplasm on the far side; in others, they appeared to terminate in its immediate vicinity. Aggregations of the precipitated Prussian blue were frequently found close to the external border of the nucleus, but with the exception of one or two instances in which a definite decision could not be made, the canaliculi did not appear to enter the structure. (See Plate 7, fig. 5; Plates 7 and 8, figs. 6, 7, 8, 9, 10, 11.)

Sometimes the canaliculi formed a fine intracellular network (see Plate 8, fig. 11); in some instances an aggregation of the deposit appeared to indicate the terminal point of a free branch. Deeply pigmented oval or round areas, varying in size, and sometimes connected with canaliculi, were occasionally met with in the course of the central lumen or in the cells, which suggested reservoirs containing an accumulation of the secretion in which the reaction had occurred. (See Plate 8, figs. 11–12.)

In certain of the parietal cells adjacent to lumina containing a large amount of the deposit, the intracellular ramifications as such were not clearly visible, but were indicated by minute isolated aggregations of Prussian blue occurring here and there in the cytoplasm. (See Plate 8, figs. 11—12.) Apart from the ramifications of the blue canaliculi and the above-mentioned indications of these, the cytoplasm was, as a rule, free from colour, but sometimes, under the most favourable daylight illumination, it appeared to be tinged with a faint, almost imperceptible, blue. In some cases it was uncertain whether this was due to a scattering of the light reflected from the material in the canaliculi.

In view of the fact previously stated (p. 69), that the presence of a free acid is a necessary factor for the production of Prussian blue *in vitro*, from the interaction of solutions of ammonium ferric citrate and potassium ferrocyanide, the occurrence of the Prussian blue reaction in the canaliculi of the parietal cells of an animal injected with a solution of these two salts affords conclusive evidence of the presence of free acid within these structures.

The evidence of the occurrence of the reaction was obtained in a very limited portion of the mucosa. The examination of a large number of sections cut from the same part of the mucosa as those which furnished the evidence of Prussian blue within the parietal cells and the lumina of the tubules afforded no proof whatever of the presence of the blue compound in the glandular structures.

This indicates the regional nature of the secretory activity, and suggests the possibility that only a very few of the gastric tubules or parietal cells are functionally active at any one time. The amount of the superficial deposit, frequently found in cases in which direct evidence of the occurrence of the reaction in the underlying tissue was not forthcoming, appears also to indicate that the Prussian blue, when formed, is removed from the tubules with great rapidity. The rapid removal of the Prussian blue, together with the variation in the functioning time of the several gland tubules, or of certain cells within these, may be largely responsible for the difficulty encountered in obtaining evidence of a positive character within the glandular structures, and for the lack of success experienced by earlier investigators.

Factors, the nature of which is unknown, are also present, which under certain conditions, equally unknown, influence the secretion of the acid in such a manner that it is no longer directed entirely towards the free surface, and consequently the Prussian blue may be found in situations other than those hitherto named.

Proof of this was obtained in two rabbits (Rabbits 5 and 6, see Table). The remarkable quantity of Prussian blue found in the case of Rabbit 5 adhering to the surface of the gastric mucosa at the end of an experiment of 6½ hours' duration has already been referred to. (For experimental data see pp. 77—79.)

In Rabbit 6, which was killed 3 hours after receiving the first, and between three-quarters of an hour and one hour after the last injection, the superficial deposit of Prussian blue was very slight. The animal had received 43 to 45 c. cms. of the injection solution by four subcutaneous injections of about 11 c. cms. each. One hour had elapsed between the first and second injections, and the remainder had been given at intervals of half-an-hour. A toxic effect was not observed. Food had been given prior to the beginning of the experiment.

The experiments with these two rabbits (Rabbits 5 and 6) although differing in some respects, both in minor details in the method of procedure and in certain of the results obtained, possess essential features in common, namely, the subcutaneous administration of a large quantity of the injection solution during the space of a few hours, and the similarity of the situation in which the Prussian blue was ultimately found in the gastric mucosa. Instead of occurring within the parietal cells, the Prussian blue was found as a granular deposit scattered on their surface remote from the lumen. The cells frequently presented the appearance of being partly covered with a delicate pale blue film, punctuated with dark blue. (See Plate 8, fig. 14.) The deposit only occurred on certain of the cells.

In many instances the surface of the cells was clear and the deposit was found in isolated areas, evidently lymphatic spaces, between the cells and close to the interstitial tissue adjacent to the external border of the tubule. In such areas, also, the dark blue granules appeared to be set in a pale blue ground substance. (See Plate 8, fig. 15.).

Certain of the intertubular lymphatic channels, as well as other lymphatic vessels adjacent to the gland tubules, were found to be lined with a deposit of Prussian blue and frequently to contain large aggregations of the blue compound. The course of the lymphatic vessels could be readily followed from their blue content. (See Plate 8, fig. 13 and fig. 16.) On the other hand, the central lumina of the gland tubules contained no Prussian blue.

Wandering cells found between the tubules, and the lymph cells in the course of the larger channels, were frequently observed to be deeply impregnated with Prussian blue, and large phagocytic cells studded with blue granules were found in the submucous coat (Rabbit 5). (See Plate 8, fig. 17.) In certain of the preparations Prussian blue was also found in the contents of the blood vessels. (See Plate 8, fig. 16.)

It is therefore evident that, under certain conditions the acid, instead of being wholly discharged into the canaliculi and thence into the central lumina of the gland tubules, may also be discharged in the inverse direction. Whether the occurrence of the Prussian blue in the lymphatic and blood-vessels thus reveals a normal tendency in the direction of the secretion or part of the secretion of the parietal cells, or whether the occurrence is due to an abnormal condition of the cell membrane brought about by one or more of the salts contained in the injection solution, can only be ascertained by further investigation.

In reference to this point, Carnot and Lelièvre,* in a recent paper, brought forward the view that the disposition of the parietal cells indicates a double physiological *rôle*, and they suggest that these cells give origin to both a digestive and an "internal" secretion. They show that the situation of the greater number of the parietal cells in the necks of the gland tubules adjoining the crypts facilitates the ready discharge of their secretion directed towards the surface, but that in addition to this the cells are in contact with the peri-tubular vascular network. They point out that this reciprocal relationship is best seen in the body of the gland tubule, where the chief cells exceed the parietal cells in number, and that in longitudinal sections, particularly in those of the stomach in which the vessels have been injected with carmine, the blood-vessels are seen to be surrounded on all sides by parietal cells, just as if the capillary constituted the central lumen of a gland. The parietal cells which thus surround a vessel belong to different gland tubules, and their intra-cellular canaliculi, demonstrated in this instance both by the Golgi-Cajal method and by iron hæmatoxylin, accordingly diverge toward three or four independent acini (dog).

These investigators further point out that this arrangement of the parietal cells in relation to the blood-vessels is very similar to that existing between the hepatic cells and the blood-vessels, and hold that it indicates an imbrication of two glandular systems, the one orientated in relation to the excretory canal, and the other in relation to the vessels. They also find it difficult to accept the idea that this concentric arrangement of the parietal cells in relation to the capillaries could have for its sole object the withdrawal from the blood of secretory products, and therefore they advance the view that the surface of contact indicates that a secretion, indiscernable histologically, is discharged in an inverse direction to that going forth from the cell to the central lumen of the tubule. They further state that evidence in support of the view of an elaboration of an internal secretion by the gastric glands, and attributable probably to the parietal cells, had already been obtained from physiological experiments in process of execution at the time of publication.

If such be the case it can be understood in the event of an upsetting of the unknown mechanism governing the direction of the digestive secretion that hydrochloric acid might then be discharged in the same direction as the internal secretion, and thus be found to be present in the lymph and blood-vessels. The richness of the blood supply in the region of the mucosa in which the parietal cells predominate has been demonstrated in the rabbit by Böhm and Davidoff.*

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* P. Carnot et A. Lelièvre, 'Compt. Rend. Soc. Biol.,' 1909, vol. 66, No. 3.

With reference to the spontaneous occurrence of the Prussian blue reaction in the skin previously mentioned, this was observed in four instances (dog, rabbit, guinea-pigs) in the muscles of the skin at the site of inoculation. The microscopical examination showed that the deposit of Prussian blue was situated in the inner structure of the muscle fibre itself. Further particulars are withheld, as the results obtained may appear in a future paper. Doubtless the explanation is that the reaction was here brought about by the presence of an organic acid, probably sarcolactic acid, resulting from injury to the fibre. Dreser,† when demonstrating the acidity of the muscles during activity by means of a decolourised solution of acid fuchsin, found the red reaction in the lymph spaces between the muscle fibres, but not in the fibres themselves.

Before passing to experiments made with other reagents, brief mention is here made of the occurrence of mitosis in the cells of the gastric glands, observed in one or two instances in the rabbit, notably in Rabbit 2. Mitotic figures in various stages were remarkably prevalent in the cells lining the crypts, and were present also in the cells of the upper part of the necks of the glands, and occasionally in some of the parietal cells in the lower portion of the tubules.

Among other observers this phenomenon has previously been observed by Sachs‡ in the surface epithelium of the gastric mucosa in man, and in the surface epithelium and in the cells of the crypts in the dog in pathological conditions; by Bizzozero§ in the cells of the crypts; and by Bensley|| in the cells of the crypts and in the chief cells of the necks of mammalian gastric glands.

A few experiments were made to see if a result approaching that recorded by Sehrwald¶ could be obtained by placing portions of the fresh gastric mucosa of the rabbit and of the guinea-pig into an aqueous solution of 2·25 per cent. ammonium ferric citrate for 18 to 24 hours, and later, after teasing, into one of 1·5-per-cent. potassium ferrocyanide. In some cases the tissues were

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* Böhm and Davidoff, 'Lehrb. d. Histol. d. Menschen,' 1895.

† H. Dreser, 'Centralbl. f. Physiol.,' 1887, vol. I.

‡ A. Sachs, 'Archiv f. exp. Path. und Pharmakologie,' 1886, vol. 22, pp. 155–173; 1887, vol. 24, p. 109.

§ Bizzozero, 'Anat. Anz.,' 1888, vol. 3, No. 26, p. 781; 'Archiv f. Mikros. Anat.,' 1893, vol. 42.

|| R. Bensley, 'Quart. Journ. Micr. Sci.,' 1899, vol. 41.

¶ Sehrwald, 'Münchener Med. Wochenschr.,' 1889, p. 177.

washed rapidly in distilled water after their removal from the solution of the double citrate and before being placed in the potassium ferrocyanide solution. The results obtained by this method, as might be anticipated, were quite of an indeterminate nature. In the case of one guinea-pig, a diffuse greenish coloration was observed here and there over the gland tubules, and, in another, a peculiar brown crystalline precipitate was observed in the parietal cells.

Table giving Results Obtained by Injecting a Solution containing Equal Parts of an Aqueous Solution of 2·25-Per-Cent. Ammonium Ferric Citrate and of 1·5-Per-Cent. Potassium Ferrocyanide.

Animal.	Mode and No. of injections.	Amount injected.	No. of hours since first and last injections.		Condition.	Toxic effect.	Prussian blue reaction—spontaneous.			Ditto, on addition of 0·5 per cent. HCl.	
							Gastric mucosa.		Skin.	Urine.	Various tissues.*
		c. cms.	First.	Last.			Surface.	Interior.			
Rabbit (1)	Intra-venous, 1 Subcutaneous, 2	10—11	35¼	2	Food given.	–	–	–	–	+	+
Rabbit (2)	Subcutaneous, 8	37	30	2½	Food given.	–	+	+	+	+ (1st day) – (2nd day)	+
Rabbit (3)	Subcutaneous, 5	31—32	25¾	2½	Starved 24 hrs.	– (1st day) + (2nd day)	+	–	–	– (Retention of urine)	+
Rabbit (4)	Intra-venous, 2	17	4½	2¾	Semi-fasting. (Starved 36 hrs. Food. Starved 24 hrs.)	–	–	–	–	+	+
Rabbit (5)	Subcutaneous, 5	50	6½	1	No food eaten during expt.	+	+	+	–	+	+
Rabbit (6)	Subcutaneous, 4	43—45	3	¾—1	Food before exp.	–	+	+	–	+	+
Rabbit (7) (sod. ferrocyanide)	Subcutaneous, 4	40	5¼	1	Semi-fasting. Food given after fast of 20 hrs. and during expt. Eaten well until after 4th injection.	+	–	–	–	+	+
Guinea-pig (1)	Subcutaneous, 2	22	3¾	1¾	Food given.	+	+	–	+	+	+
Guinea-pig (2)	Subcutaneous, 2	16	5¼	3	...	+ (Polyuria)	+	–	–	+ – ?	+
Guinea-pig (3)	Subcutaneous, 2	19	6¾	4½	...	+ (Polyuria)	+	–	+	+	+
Dog (1)...	Subcutaneous, 2	55	3¼	1¼	Starved 48 hrs. Food given 1 hr. before expt.	+ (Vomiting)	–	–	–	–	+
Dog (2)...	Subcutaneous, 2	42	3¼	...	...	+ (Comatose)	+	–	+	+	+

+ means positive result. – means negative result.

* After their removal from the body, and subsequent exposure to the action of dilute hydrochloric acid, the Prussian blue reaction developed, at various times, in the following tissues:—Bladder, kidney, liver, small and large intestines, ovary, fallopian tubes, uterus, vagina, and lymph gland of axillary region (dog). The kidney became quite blue in many cases, and under the microscope the uriniferous tubules presented a very striking appearance.

In view of the proof obtained of formed acid existing in the canaliculi of the parietal cells, it was a matter both of interest and of importance to repeat the experiments of Mary Greenwood* and of Macallum,† referring to the presence of chlorides in the cytoplasm of the parietal cells, and, if possible, thereby to increase collateral evidence of the elaboration of the hydrochloric acid of the gastric juice within these cellular elements of the gastric mucosa.

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* M. Greenwood, *ibid.*

† A. B. Macallum, 'Ergebn. d. Physiol.,' 1908, vol. 7, p. 628.

As before stated (p. 68), owing to the fact that, on exposure to sunlight, the phosphate and the carbonate of silver form coloured reduction compounds similar in colour to that of the chloride, the results obtained by Mary Greenwood by the use of silver nitrate furnished only a partial proof of the presence of an excess of chlorides in the parietal cells as compared with the chief cells, but definite proof of this was afforded by Macallum, who eliminated the error which could arise in the interpretation of the results obtained by the silver nitrate method as used by the earlier investigator, by the addition of free nitric acid to the silver nitrate solution, which permits the formation of the coloured reduction compound from chlorides, but prohibits a similar formation from phosphates and carbonates.

Macallum* also pointed out that, in the presence of free nitric acid, organic substances, with the exception of taurine and creatine, will not give the colour reaction similar to that of the sub-chloride of silver, and that proteids and albuminoids, when freed from all traces of haloids, do not give any compound of silver that will reduce under the action of light.

In the present series of experiments, the methods used were similar to those employed for the determination of the distribution of the chlorides in nerve-cells and fibres by Macallum and Menten,† and of the phosphates in muscle by Maud Menten.‡ For the detection of salts, such as chlorides, phosphates, and carbonates, giving a coloured reduction compound of silver on exposure to sunlight, a deci-normal solution of silver nitrate was used; and, for demonstrating the chlorides alone, a deci-normal solution of silver nitrate containing 1·5 per cent. free nitric acid. The silver solution was in each case made with pure water.

Investigations were carried out on the fresh gastric mucosa of the rabbit and of the guinea-pig, the animals having been killed beforehand by the methods described previously (p. 74) for the two species named. In each case portions of the fresh stomach, washed quickly in distilled water, were rapidly cut into sections, and the mucosa detached from the outer layers of the stomach wall placed in vessels containing the respective solutions. The tissues were then carefully teased with goose quills, to permit the reagent to penetrate. Penetration does not take place readily, and, although the presence of the free nitric acid aids the diffusion of the nitrate in the solution which contains the acid, careful teasing is necessary, whether nitric acid is present in the solution or not. After allowing the respective solutions to act upon the tissues for a certain length of time, the vessels containing them were then exposed to sunlight, or to as bright a light as was available, for several days; and when the maximum reaction had been obtained, the tissues were mounted in 50-per-cent. glycerine; or, after being exposed to light for some time, portions of the tissues were mounted in 50-per-cent. glycerine, to which a few drops of the reagent were added, and again exposed to sunlight in this medium.

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* A. B. Macallum, 'Roy. Soc. Proc.,' 1905, B, vol. 76, p. 217.

† A. B. Macallum and Maud L. Menten, 'Roy. Soc. Proc.,' 1906, B, vol. 77, p. 165.

‡ Maud L. Menten, 'Canadian Inst. Trans.,' 1908–1909, vol. 8, p. 414.

The characteristic reaction was obtained in the tissues acted upon by each of the solutions, and drawings were made of specimens mounted in 50-per-cent. glycerine. Fig. 18 shows the appearance of a tubule treated with the silver nitrate solution without acid, and figs. 19 to 22 the appearance of tubules after treatment with silver nitrate solution containing free nitric acid.

It will be seen that in both cases the parietal cells are differentiated from the chief cells, but since the "reduced" phosphate and carbonate compounds of silver are brown in colour, and as the sub-chloride, although violet, reddish violet, or bluish violet when in mass, may also be reddish brown, as shown by Macallum,* when it occurs in thin layers or deposits, the shade apparently depending on the existence of the compound in a finely divided state or otherwise, it is clear that it would be impossible to make a definite statement as to the presence of an excess of chlorides in the parietal cells in the tubule depicted in fig. 18, because the brown colour could also be attributed to the precipitation of the "reduced" phosphate or carbonate. Such a statement can, however, safely be made with regard to the reaction shown in figs. 19 to 22, for in these cases, owing to the differentiating action of the free nitric acid present in the silver nitrate solution, the reaction can only be due to the precipitation of the sub-chloride of silver.

The intensity of the reaction obtained in the parietal cells of both the rabbit and the guinea-pig, and the marked manner in which the parietal cells stand out *in situ* in the tubules of the rabbit shown in figs. 19, 20, due to the chlorides being more abundantly present in these cells than in the chief cells, are results which fully corroborate those obtained by Macallum,† when using the same method and the same animals, and furnish evidence of the source of the hydrochloric acid in the parietal cells, and also give support to the view that this is elaborated in the cytoplasm of these cells.

With the hope of establishing the presence of the acid in the cells by more than one method, some experiments were also made with Tropæolin OO

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* A. B. Macallum, 'Roy. Soc. Proc.,' 1905, B, vol. 76, p. 217.

† A. B. Macallum, 'Ergebn. d. Physiol.,' 1908, vol. 7, p. 628.

(*cf.* p. 63), but the results obtained were of an indefinite nature, although indicating the presence of acid. The rabbit and the guinea-pig were the animals used. In the experiment with the rabbit, the animal was killed by chloroform, and portions of the gastric mucosa (fresh) were teased in a saturated aqueous solution of tropæolin, or in a saturated glycerine solution (25 or 50 per cent.). A red colour was observed in the region of the mucosa containing the peptic glands.

With the guinea-pig, the tropæolin was injected subcutaneously as a saturated solution made up in either distilled water or in Ringer's solution. The amount injected varied from 18 to 22 c. cms., and was administered by two injections in the abdominal region, at intervals varying from 1 to 1½ hours, or from 2 to 2½ hours. The duration of the experiment varied from 4 to 5½ hours. The reagent, both in aqueous solution and when dissolved in Ringer's fluid, exerted a decidedly toxic influence, and its presence in the body appeared to cause the animals considerable discomfort. The contents of the stomach were in a very watery condition, and much gas was present both in the stomach and intestines. Inflammatory conditions supervened at the site of inoculation. The urine on some occasions gave the characteristic rose-coloured reaction on the addition of concentrated hydrochloric acid, but not on the addition of a 0·5 per cent. solution. Death was produced by shock. On opening the stomach a rose-coloured area was observed on the surface of the mucosa near the œsophageal opening and in the region corresponding to the border of the lesser curvature. In the guinea-pig injected with the reagent dissolved in Ringer's fluid, a rose colour was also observed in the pyloric region. It extended across the mucosa of the superior half of the organ in this region, and was visible also on the surface of the mucosa of the duodenum adjacent to the orifice of the pylorus. Portions of the gastric mucosa were teased and examined in 50 per cent. glycerine without delay, but owing to the general distribution and diffuseness of the rose colour in the gland tubules, no positive information as to the occurrence of the acid within any one type of cell was obtained by this method.

The drawings accompanying the paper were made with the aid of an Abbé camera lucida (Zeiss or Leitz) with a Zeiss microscope, compensating ocular No. 4 (Zeiss) or No. 2 (Leitz), and Leitz objectives No. 3 or one-twelfth oil immersion. The drawing paper was laid flat on the table or on a board placed on a level with the stage of the microscope. The drawings illustrating the results obtained in the Prussian blue experiments were mostly made from unstained preparations. In some cases, illustrations of which have not been included, the sections were stained with a 0·5-per-cent. aqueous solution of neutral red. Previous to immersion in the staining fluid, the sections were carefully washed in distilled water to remove the alcohol used for fixation, to prevent the yellow coloration which would otherwise have resulted on the application of the dye. With the exception of the nuclei staining a pink colour of moderate intensity, the parietal cells remain uncoloured by this method; they are, therefore, well differentiated from the chief cells, which are stained crimson. In a vertical section of that part of the gastric mucosa in which the parietal cells are most numerous, the neck region of the gland tubules appears as a clear band, as the surface epithelium and the cells of the crypts are also stained crimson.

From the results obtained in the gastric mucosa of the rabbit previously injected with a solution containing equal parts of an aqueous solution of 2·25-per-cent. ammonium ferric citrate and of 1·5-per-cent. potassium ferrocyanide, it may be said in conclusion that the occurrence of Prussian blue, not only in the lumina of the gland tubules, but also in the canaliculi in the parietal cells, makes it certain that the hydrochloric acid is, at least, already formed and free in the secretion as it appears in the canaliculi. Whether the acid, or, more correctly speaking, whether its hydrogen ions occur in the cytoplasm of the cells still remains an open question, although the very faint blue occasionally observed in a parietal cell seems to postulate an affirmative. It is, moreover, to be noted that if the acid is formed and set free in the canaliculi, then the cytoplasm lining the latter must constitute a membrane permeable to the H and Cl ions, but not to $\overset{+}{\mathrm{Na}}$, $\overset{+}{\mathrm{K}}$, $\overset{-}{\mathrm{PO}}_4$, or $\overset{-}{\mathrm{CO}}_3$. If that were entirely the case, no free acid should occur in the lymphatics besides the tubules. The acid here can only be derived from the cytoplasm of the parietal cells, and if the latter have absorbed it from their canaliculi, then it would be difficult to explain why the cytoplasm does not neutralise the acid, for the removal of the hydrogen ions from the parietal cells must make their cytoplasm distinctly alkaline. If, to overcome this difficulty, we suppose that the cytoplasm is so constituted as to maintain the ionisation of the hydrogen and the chlorine in the presence of alkalies, we attribute a property to it that could be also responsible for the formation of the acid in the first place in the cytoplasm.

The occurrence of free acid in the canaliculi, and also, in certain cases, in the lymphatic vessels between the gastric tubules, is, therefore, best explained by supposing that the cytoplasm of the parietal cells forms in itself the free acid, and, under ordinary conditions, that the free acid so formed diffuses into the canaliculi, but, under certain conditions, the character of which is unknown, the cell loses the sense of direction, so to speak, and, as a consequence, the free acid diffuses in the opposite direction, that is, out, into the inter-tubular tissue.

The source of the hydrochloric acid in the parietal cells is, without doubt, the chlorides, present, as already shown, in greater abundance in them than in the chief cells or other adjacent tissue elements. The results of observations with Macallum's cobalt sodium hexanitrite method for demonstrating potassium* have not yet shown that the latter element occurs more abundantly in the parietal than in the chief cells, consequently the chlorine demonstrable in the parietal cells must be associated chiefly, if not entirely, with sodium.

Rosemann† made analysis of the gastric juice of the dog, and, calculating from his results, it was found that in the ash the proportion of the potassium to the sodium was 1 : 1·23 and 1 : 2·17, whereas in the blood plasma the proportion is 1 : 0·0685. Rosemann consequently concluded that the acid arises not through simple diffusion out of the blood, but from the bodies of the cells concerned. As the amounts of these elements in the gastric juice are very small ($K = 0{\cdot}03077$–$0{\cdot}04328$, and $Na = 0{\cdot}01979$–$0{\cdot}02502$), and, further, as the percentage of potassium in the juice does not greatly exceed that of potassium in the blood plasma (= 0·025 per cent.), it is possible that, though the parietal cells take up the two alkaline chlorides from the blood plasma and lymph, yet in the formation of the acid they utilise only the sodium chloride, the sodium being retained to pass back into the blood and lymph. If potassium chloride constituted a source of the chlorine of the acid in the gastric juice, the potassium in the latter would have, as in the case of sodium, undergone relatively a great diminution in amount.

In conclusion, I desire to express my grateful thanks to Prof. A. B. Macallum, at whose suggestion this work was undertaken, for his supervision and for affording me the facilities for carrying out the research in the Bio-Chemical Laboratory of the University of Toronto, and, in particular, I wish to acknowledge my indebtedness to him for his valuable help in the chemical matters connected with the investigation, and with the physico-chemical interpretation of the results.

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* A. B. Macallum, 'Journ. Physiol.,' 1905, vol. 32, p. 95.

† Rosemann, *loc. cit.*, pp. 522—523.

EXPLANATION OF PLATES.

Figs. 1–17 represent results obtained in the gastric mucosæ of rabbits injected subcutaneously with a solution containing equal parts of an aqueous solution of 2·25 per cent. ammonium ferric citrate and 1·5 per cent. potassium ferrocyanide.

PLATE 7.

Fig. 1.—Opened stomach of Rabbit 5 fixed in absolute alcohol. Natural size. Drawing shows deposit of Prussian blue adhering to surface of mucosa of dorsal wall and on that of unremoved portion of ventral wall (pyloric region). Maximum reaction as observed on surface.—Note that Prussian blue is distributed in region of œsophageal opening and in adjacent region of lesser curvature, and that in this instance deposit also occurs in pyloric region.

Fig. 2.—Vertical section of œsophageal region of stomach fixed in absolute alcohol, showing deposit of Prussian blue in central lumina of gland tubules, in crypts, and on surface of mucosa. (Rabbit 2, 30 hours after the first injection.) ×96.

Fig. 3.—Gland tubules of gastric mucosa in which lumina are lined with granular deposit of Prussian blue. The irregularly occurring aggregations of deposit cause lumina to present a wavy, jagged appearance. Two side branches can be traced passing to a cell; within other cells small aggregations of deposit are seen. ×1100.

Fig. 4.—Gastric tubule showing deposit of Prussian blue in central lumen, in side branch, and in canaliculi resulting from its bifurcation. ×1100. (Rabbit 2.)

Fig. 5.—Parietal cell penetrated by two side branches connected with lumen and containing Prussian blue. ×1100. (Rabbit 2.)

Figs. 6, 7, 8, 9.—Parietal cells, the canaliculi within which contain Prussian blue. Note larger side channel, ramification of fine canaliculi in the cytoplasm of cells, their wavy course, frequent proximity to nucleus, and beaded appearance due to isolated aggregations of deposit within. Figs. 6–9, × 1100. (Rabbit 2.)

PLATE 8.

Fig. 10.—Parietal cells with Prussian blue in canaliculi. ×720. (Rabbit 2.)

Fig. 11.—Gland tubules cut tangentially. Along the course of one of the central lumina and of a side channel, lined with Prussian blue, deeply stained oval enlargements occur, suggesting reservoirs containing accumulation of secretion. In one of the parietal cells canaliculi are seen in form of a network. ×1100. (Rabbit 2.)

Fig. 12.—Gland tubule. Prussian blue seen to be irregularly deposited along course of central lumen and to be present in side branch. An oval area suggesting a reservoir containing accumulation of secretion is seen, and small isolated aggregations of deposit within cells. ×1100. (Rabbit 2.)

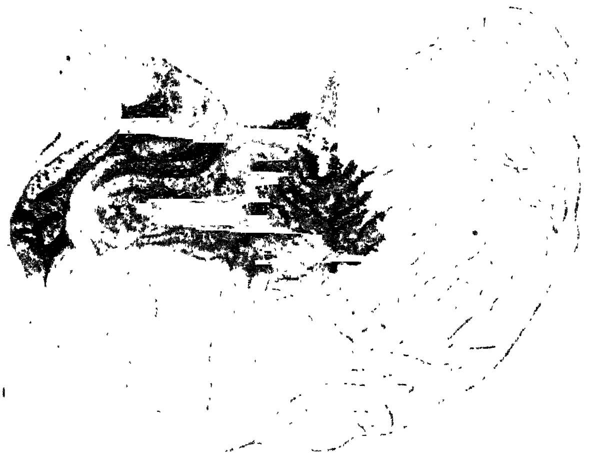
Fig. 13.—Intertubular lymph channel containing Prussian blue. ×920. (Rabbit 6.)

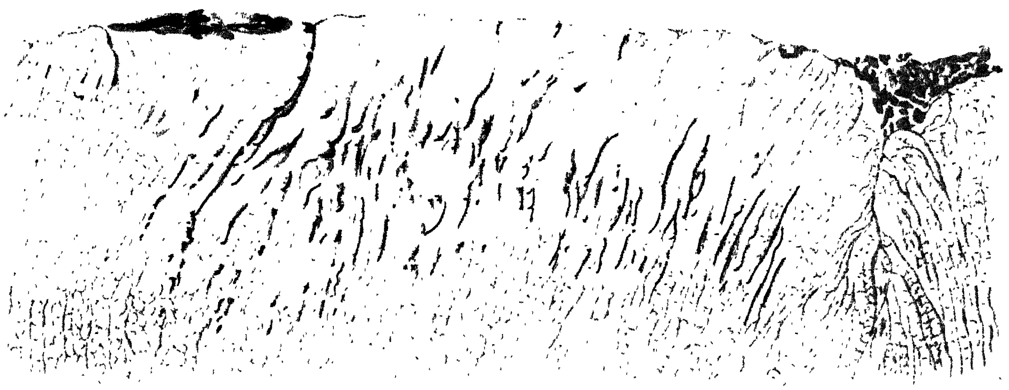
Fig. 14.—Granular deposit of Prussian blue in a film of blue on surface of cells and in adjacent lymph space. ×920. (Rabbit 5.)

Fig. 15.—Intercellular lymph space containing granular deposit of Prussian blue in a pale blue ground substance. ×640. (Rabbit 5.)

Fig. 16.—Transverse section of gastric mucosa. Prussian blue is seen in an interglandular lymph vessel, and mixed with contents of blood vessel. ×640. (Rabbit 6.)

Fig. 17.—Phagocytes in sub-mucous coat of stomach, which have taken up the granular deposit of Prussian blue. ×800. (Rabbit 5.)





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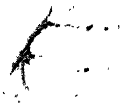

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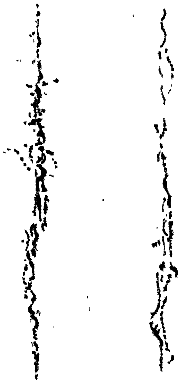

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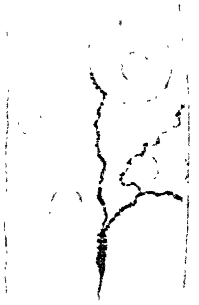

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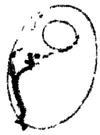

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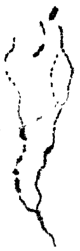

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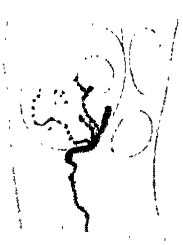

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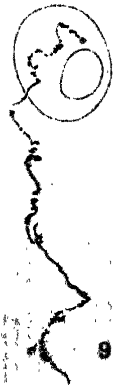

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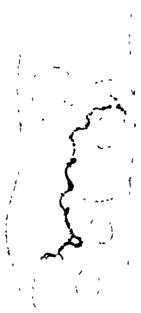

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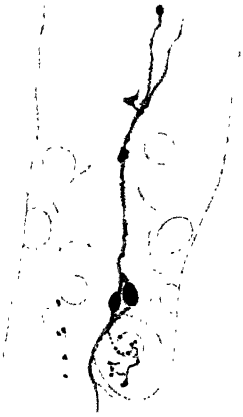

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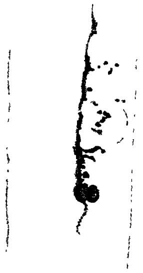

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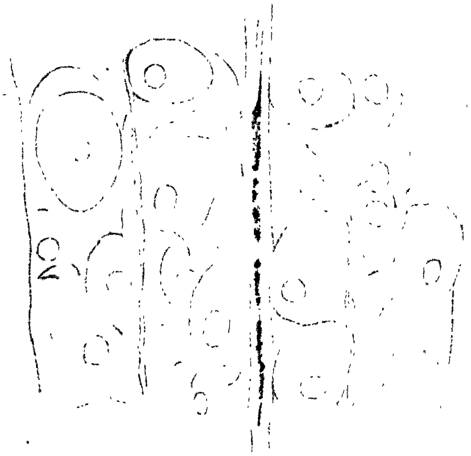

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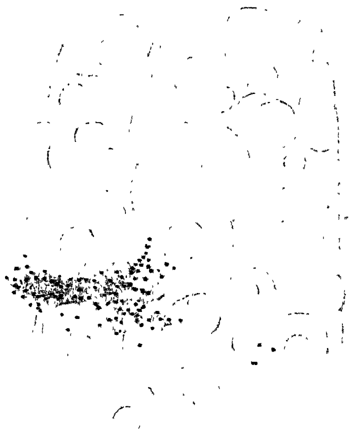

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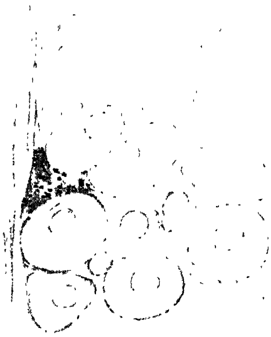

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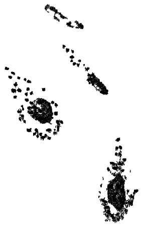

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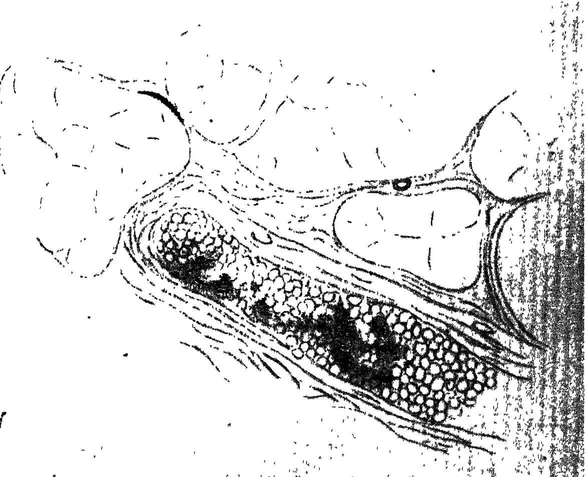

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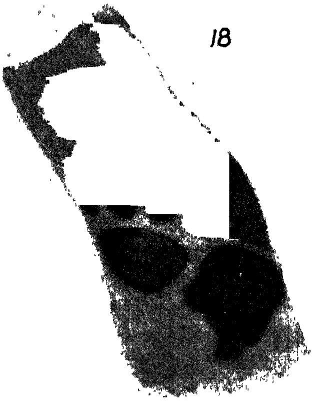

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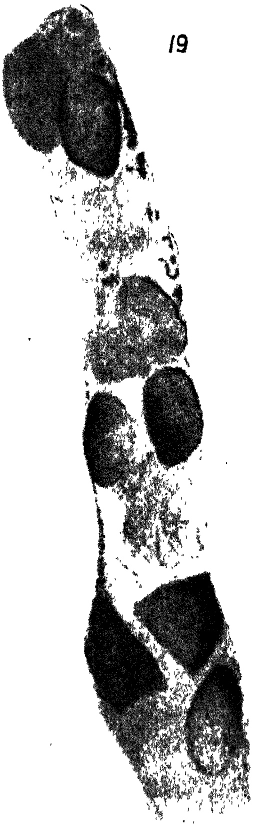

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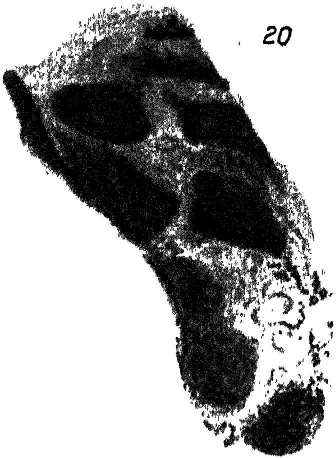

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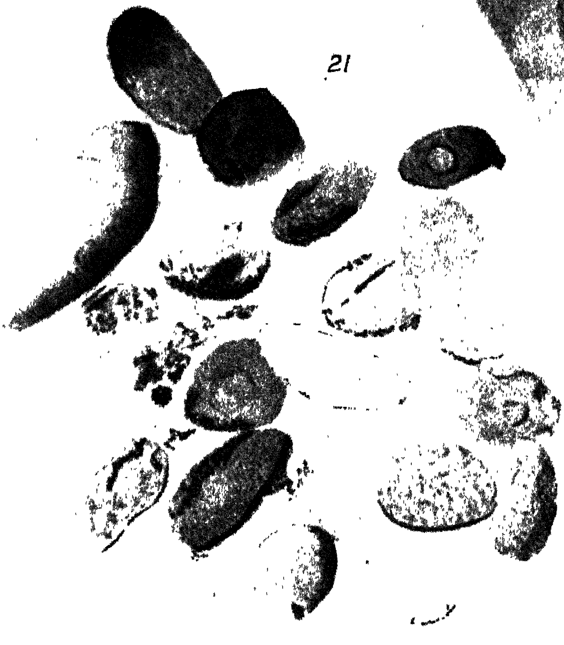

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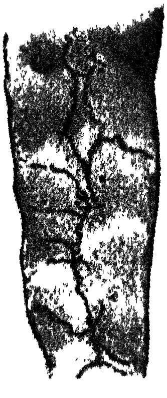

Grout, sc. & imp.

Plate 9.

Figs. 18–22 represent results obtained by action of silver nitrate on teased gastric glands of rabbit and guinea-pig.

Fig. 18.—Portion of the gastric gland of a rabbit exposed to the action of $N/10\ AgNO_3$ for two days. The parietal cells are differentiated from the chief cells by their brown colour, but in this instance the brown reaction may be due to presence of phosphates, carbonates, or chlorides, or to all three of these "reduced" compounds of silver. × 690.

Fig. 19.—Portion of teased gastric gland tubule from œsophageal region of normal rabbit, treated when fresh with $N/10\ AgNO_3 + 1{\cdot}5$ per cent. HNO_3. Parietal cells are differentiated from chief cells by the intensity of their brown coloration. The reaction in this case is due to precipitation of "reduced" chloride of silver; its occurrence in such marked degree demonstrates the presence of greater abundance of chlorides in these cells than in the chief cells. × 700.

Fig. 20.—Base of gastric tubule from œsophageal region of normal rabbit, treated when fresh with $N/10\ AgNO_3 + 1{\cdot}5$ per cent. HNO_3. Excess of chlorides again demonstrated in parietal cells by their brown coloration. (See explanation of fig. 19.) ×690.

Fig. 21.—Teased preparation of gastric gland tubules treated with $N/10\ AgNO_3 + 1{\cdot}5$ per cent. HNO_3. Abundance of chlorides in parietal cells is demonstrated by their reddish-brown colour. ×840. Guinea-pig.

Fig. 22.—Teased preparation of gastric gland tubule from fundus region of normal guinea-pig, treated with $N/10\ AgNO_3 + 1{\cdot}5$ per cent. HNO_3, showing presence of chlorides in deposit in lumen and in intercellular and intracellular channels. × 640.

The Effect of Gravity upon the Movements and Aggregation of Euglena viridis, *Ehrb., and other Micro-organisms.*

By Harold Wager, F.R.S.

(Received August 2,—Read November 17, 1910.)

(Abstract.)

In the course of some experiments on the effect of various physical forces upon the movements of *Euglena viridis,* it was found that, when placed in the dark in shallow vessels or narrow tubes, a peculiar aggregation of these organisms takes place into network-like patterns or more or less well-defined circular groups. The aggregation is only visible when the Euglenæ are abundant, and is more regular and pronounced in a shallow vessel than in a deep one, especially if it is completely filled with the liquid containing the organisms, and sealed up so that the upper surface is not in contact with the air. If a narrow tube filled with water containing sufficient Euglenæ to give it a pronounced green colour is placed horizontally in the dark or in a weak light, the aggregation takes the form of a series of nearly equally spaced groups, like green bands, crossing the tube from one side to the other, and extending along its whole length.

Each group shows clearly two distinct regions, a central denser one consisting of cells moving downwards, and a lighter peripheral area consisting of cells moving more or less regularly upwards. There is, in fact, a constant cyclic movement downwards and upwards, which is kept up so long as the aggregation persists. Examination with a pocket lens shows that, as the organisms reach the bottom of the stream, they gradually separate from one another, and begin to move upwards. As they reach the upper surface, they are seen to be drawn towards the central denser region of the group, and again enter the downward stream.

This aggregation, with its regular cyclic movements, may persist for several days, until the Euglenæ die, in fact, provided they are kept in the dark or under red glass. In a good light the aggregation soon disappears, but reappears again in a few seconds when placed in the dark. So also, if the aggregation is caused to disappear by shaking the vessel in which the Euglenæ are contained, it reappears again immediately the disturbance has ceased.

The ease and rapidity with which the aggregation takes place appear to depend, among other things, upon the activity of the Euglenæ. Anything

which tends to inhibit this, and to cause sluggish movements, such as a very low temperature, may entirely prevent the aggregation; but so long as the slightest movement takes place in the organisms, an aggregation may be visible, although it may be very indistinct, and only recognisable as a delicate ripple-mark like effect.

The aggregation does not appear to be due to any marked extent, and probably not at all, to currents set up in the liquid either by heat or evaporation; and it appears not to be dependent upon the presence or absence of oxygen or carbon dioxide, except in so far as these may be necessary to maintain the motility of the organism.

The downward movement appears to be a purely mechanical one, dependent upon the specific gravity of the organism, and is not due to a stimulus which evokes a physiological response as in geotropism or geotaxis. The upward movement is, on the other hand, due partly to the activity of the organisms themselves, partly no doubt to the upward currents set up in the liquid by the friction of the downward moving stream. The upward movement of *Euglena* appears to be controlled, so far as the orientation of its elongate body is concerned, by the action of gravity. If not crowded together, the motile cells of *Euglena* may move in any direction in space, but if anything interferes with their movements the pull of gravity immediately causes them to take a more or less vertical position with the posterior end pointing downwards. This is due to the fact that the organism is heavier than water and that the posterior end is heavier than the anterior.

The network-like aggregations and groupings resemble very closely in many respects the cohesion figures which are formed, under certain conditions, when fine sediments of various kinds, such as precipitates of manganese dioxide, osmium dioxide, etc., are allowed to settle slowly in a liquid; and the conclusion has been arrived at that such aggregations are probably of the nature of cohesion figures, due to the action of gravity upon organisms massed together, combined with the vortical movements set up when the streaming movements begin to take place.

In addition to *Euglena viridis,* experiments were made with colourless forms of the same species, with *E. deses, Chlamydomonas, Volvox,* Bacteria (*Spirillum*), and one of the fresh-water Peridineæ, in all of which the phenomena were visible, but with considerable variation in the extent to which it was produced.

It is a remarkable fact that the movements of micro-organisms should be controlled in this purely mechanical fashion, but the advantage to those species which, like *Euglena,* are often found in a confined space in very large numbers must be very great, as by its means a constant circulation of them through all parts of the liquid takes place, and they are prevented from accumulating in such dense masses as would be detrimental to their existence by interfering with their assimilatory or respiratory functions. It is possible also that some of the peculiar phenomena of plankton distribution may be explained in this way.

Some Investigations Dealing with the State of Aggregation of Matter.—Parts I–III.

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(Communicated by Prof. E. H. Starling, M.D., F.R.S. Received August 12,—
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Part I.—*On the Action of Salts in Heterogeneous Systems, and on the Nature of the Globulins.*

A. General Theory and Results.

During the course of some investigations on the action of formaldehyde on the proteins, the observation was made that this aldehyde, when added to an aqueous solution of Witte's peptone, produces a precipitate, and that the reaction could be either partially or completely inhibited by the presence of neutral salts. This phenomenon was also noticed some years ago by T. Sollman,* who offered no satisfactory explanation of the facts. The more recent investigations of Sörensen† have shown that when formaldehyde reacts with amino-acids a methyleneimino-derivative is produced, which is readily hydrolysed in the presence of water, yielding the original amino-acid and formaldehyde. The reaction is therefore a reversible one, and can be represented by the general equation

$$(NH_2)R{\cdot}COOH + HCHO \rightleftharpoons (CH{:}N)R{\cdot}COOH + H_2O.$$

The amino-acid is only completely converted into the methyleneimino-derivative in the presence of a large excess of formaldehyde, and the methyleneimino-acid thus produced is, in contrast to the amino-acid from which it was formed, so strongly acid that it can be titrated with caustic alkalis in the presence of phenolphthalein as indicator. These results are an extension and application of those which had been obtained some years before by Schiff.*

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* 'American Journal of Physiology,' 1902, vol. 7, p. 220.

† 'Biochem. Zeitsch.,' 1907, vol. 7, p. 45.

It was noticed that when formaldehyde was added to Witte's peptone, and the reaction mixture was then titrated by the Sörensen method, the same amount of alkali was required for neutralisation both in the presence and absence of salts, *i.e.* whether the precipitate was formed or not. In both cases, therefore, the formaldehyde had entered into reaction with the amino-groups in the peptone. A clue to the nature of the phenomenon was afforded, however, by Schiff's observation that asparagine, when treated with formaldehyde, yields a methyleneimino-derivative, which readily undergoes polymerisation or condensation in which more than one molecule takes part, with the formation of a complex insoluble compound.† It was therefore conceivable that certain constituents of Witte's peptone react in a similar way, and the experiments recorded in the succeeding communication indicate that the precipitate is formed from the most complex polypeptide constituents of the peptone. If these be sufficiently complex as to be of colloidal nature, and to yield a solution which acts as a heterogeneous system, it is conceivable that adsorption of salts takes place on the surface of the colloidal molecules, and thus, by a kind of sterical inhibition, prevents the reaction of the large molecules with one another, to form, in the case under consideration, highly complex polymers.‡

The inhibitory action of salts on the polymerisation or condensation of the methyleneimino compounds was studied quantitatively in some detail (see succeeding paper), and found to agree very closely (with certain exceptions, which can be readily explained) with their inhibitory action on the formation of the zinc protein compounds which are produced when zinc sulphate is added in small quantities to protein solutions.§

During the course of these investigations it was also noticed that a marked parallelism existed between the inhibitory action of salts on the formation of the precipitate produced when formaldehyde is added to Witte's peptone and their capacity for dissolving the globulins, and this analogy suggested an explanation of the chemical character of this class of proteins, and of the fact that they are soluble in certain salt solutions, and insoluble in water.

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* Liebig's 'Annalen,' 1901, vol. 319, p. 287.

† Liebig's 'Annalen,' 1899, vol. 310, p. 25.

‡ Instances of sterical hindrance of reaction by atoms or groups combined in a molecule are, of course, well-known in the chemistry of simpler organic compounds, and are dealt with in detail in text-books on stereochemistry. (*Cf.* Stewart, 'Stereochemistry,' pp. 314–443.

§ The conditions of the reaction between zinc sulphate and protein solutions are somewhat complex and have been studied by Pauli (Hofmeister's 'Beiträge zur Physiol. u. Pathol. Chem.,' 1905, vol. 6, p. 233). He found that when egg-white solution of a given strength was mixed with zinc sulphate solution of varying concentrations, two maxima of precipitation occurred. The amount of precipitate gradually increased when the concentration of the zinc salt rose from 0·001 to 0·05 normal. As the concentration increased beyond this point, the amount of precipitate formed gradually diminished until a concentration corresponding to that of a normal solution was attained. At this point

From general considerations of the chemical characters of proteins and their hydrolysis products, it is conceivable that substances of this class can be either basic or acid in their nature, or that they can be truly amphoteric, and combine both acid and basic properties. As members of the basic class, which yield on hydrolysis chiefly diamino-derivatives, the protamines may be cited as examples. Other proteins of distinctly acid character are also known, in which class caseinogen and other phosphoproteins may be included, although such substances belong, strictly speaking perhaps, to the class of conjugated proteins. The investigations of Hardy on serum globulin,* and of Osborne on edestin,† have shown that the globulins have both acid and basic functions. If it be assumed that globulins contain both basic and acid groups in the molecule, and so situated stereochemically that internal neutralisation between these groups cannot take place, then it is possible that two molecules can enter into reaction with one another forming a complex according to the scheme—

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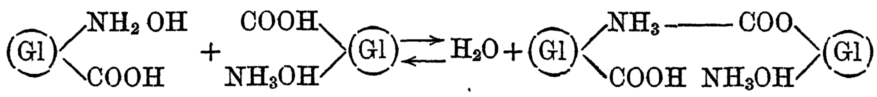
no precipitate whatever was produced, and no further precipitation occurred until the concentration of the zinc salt was twice normal. The precipitate formed at the lower limits differed from that formed at the upper limits in that the former was irreversible (*i.e.* did not redissolve on diluting or concentrating the solution), whereas the latter was reversible. The precipitate formed at the lower limits is that of the zinc salt of the protein, whereas that formed at the upper limits is the ordinary protein precipitation by salts. The following is suggested as a possible explanation of this action of zinc salts on proteins. If the carboxyl groups in the protein molecule are situated so far apart that the salt of the dyad metal cannot be formed by replacement of the hydrogen from two carboxyl groups of the same protein molecule, the zinc salt must be formed by the replacement from carboxyl groups from two molecules of protein, and be of the type $Zn\,(Alb)_2$. This would be a larger aggregate than the original protein molecules. If the zinc salt could be adsorbed, as well as enter into chemical reaction in the way described, it is conceivable the adsorbed molecules would surround the protein molecules and inhibit the chemical reaction in which two molecules of protein and one of zinc salt take part. The phenomenon of the inhibition of the formation of zinc protein derivatives by excess of zinc sulphate is, if the explanation suggested above is correct, analogous to the inhibition of formation of complex methyleneimino-peptones, with the difference that the adsorbed salt, in addition to its inhibitory action, can also take part in the chemical reaction by means of which the formation of larger aggregates is brought about. Other similar irregular groups ("unregelmässige Reichen") of colloidal reactions have been described by Neisser and Friedmann ('Münchener Med. Wochenschr.,' 1903, No. 11) and Bechhold ('Zeitsch. Physikal. Chem.,' 1904, vol. 48, p. 355), and others. (For general discussion of these phenomena see 'Freundlich. Kapillarchemie,' Leipzig, 1909, p. 462.)

* 'Journal of Physiology,' 1905, vol. 33, p. 251.

† 'Zeitsch. Physiol. Chem.,' 1901, vol. 33, p. 240.



$$(\mathrm{Gl})\begin{matrix}\diagup\mathrm{NH_2\,OH}\\ \diagdown\mathrm{COOH}\end{matrix}+\begin{matrix}\mathrm{COOH}\diagdown\\ \mathrm{NH_3OH}\diagup\end{matrix}(\mathrm{Gl})\rightleftharpoons\mathrm{H_2O}+(\mathrm{Gl})\begin{matrix}\diagup\mathrm{NH_3}\text{———}\mathrm{COO}\diagdown\\ \diagdown\mathrm{COOH}\quad\mathrm{NH_3OH}\diagup\end{matrix}(\mathrm{Gl});$$

i.e. neutralisation can take place between the acid group of one globulin molecule and the basic group of another. If either the acid or basic functions, or both, be sufficiently well marked, the product formed by the combination of two molecules will be stable, and will undergo but little hydrolysis in the presence of water (*i.e.* the reaction in the above equation will proceed nearly to completion in the direction indicated by the arrow pointing to the right). The nature of the globulins can be explained by assuming that the undissolved globulin is a complex of this type, which in the presence of water undergoes but little hydrolytic dissociation. That a certain amount of hydrolysis does take place, however, is indicated by Hardy's observation that after repeated washing of serum globulin by water, the washings have always a higher electrical conductivity than the wash water. If salts be added to the water and adsorption on the surface of the dissociated globulin takes place so that it is stereochemically inhibited by the adsorbed salt molecules from forming aggregates by salt formation with other molecules, then the equilibrium between the solid undissociated globulin and water will be disturbed; the more rapidly the salt molecules are adsorbed, the further will reaction proceed in the direction of the arrow pointing to the left in the above equation, and the more the globulin will appear to be dissolved (or to speak perhaps more correctly, to be disaggregated). In offering this explanation as to the nature of the globulins one assumption has been made, viz. that in aggregation under "chemical stimulus" (*i.e.* salt formation) the molecule reaches such large dimensions that it is no longer capable of forming even colloidal solutions, but becomes insoluble. This assumption is supported, however, by certain experimental facts demonstrated by Hardy in the case of serum globulin and by Osborne in the case of edestin.* In their determinations of the solubility of globulins in acids and bases, it was found that the same solvent capacity was possessed by equimolar and not by normal solutions (*i.e.* $HCl = H_2SO_4 = H_3PO_4$; $NaOH = Ba(OH)_2$). This fact can be explained by assuming that in the case of the polybasic acids only the acid salts of the type $(\mathrm{Gl}')\cdot\mathrm{HSO_4}$ are soluble, and the neutral salts of the type $(\mathrm{Gl}')_2\mathrm{SO_4}$ are insoluble; similarly, salts of the type $(\mathrm{Gl}'')_2\mathrm{Ba}$ are also insoluble. In both these latter cases it may be assumed that the molecule has reached such large dimensions that no kind of solution can take place.

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* *Loc. cit.*

The correctness of the conceptions advanced above as to the nature of the formaldehyde-peptone reaction, and of the globulins, receives support in the results obtained in a general study of the action of salts in heterogeneous systems, in which an attempt was made to correlate their action in such systems with the physical properties of their solutions.

The well-known deduction made by Willard Gibbs from thermodynamical considerations, that substances which reduce the surface tensions of solutions tend to accumulate at the surface, which was expressed by the formula

$$-u = \frac{R}{CT}\frac{d\sigma}{dc}$$

(where R is the ordinary gas constant, T the absolute temperature, u the excess of molecules accumulating at the surface, c the concentration and σ the surface tension) has been repeatedly applied in late years to the study of adsorption, notably by Donnan, and by Freundlich, and the above formula has at the suggestion of the former been quite recently submitted to experimental verification by Lewis.* From these considerations it might be deduced that the amount of adsorption taking place from salt solutions would be a function of the surface tension of these solutions, the greater adsorption taking place from those solutions possessing a low surface tension. If the suggestions advanced above as to the nature of the formaldehyde-peptone reaction and of the globulins are correct, it should follow that the inhibitory action of salts on the precipitate formation in the former system, and their solvent (or disaggregating) capacity in the latter, are functions of their surface tension.

This hypothesis has been submitted to experimental test, and the results obtained are given in detail below. It was found that in salts of the same series, the lower the surface tension, the greater was the solvent capacity for globulins, and the greater the inhibitory action on the formation of the insoluble methyleneimino-peptone derivative. This general result, however, was found, as already stated, *only when salts of the same series were compared.* The inorganic sodium salts, for example, were found as a general rule to have greater disaggregating capacities than the organic sodium salts having the same surface tension. Sodium salicylate, furthermore, had a far greater disaggregating capacity than sodium benzoate, a salt with very nearly the same surface tension. Sodium formate occupied a position intermediate between the organic and inorganic salts. From these results it appeared as if some property of salt solutions other than their surface tension was concerned in their disaggregating capacity, and the clue as to this property was afforded by certain generalisations on the rate of action in heterogeneous systems, first put forward by Noyes and Whitney,* to explain the rate of solutions of solids in liquids, and subsequently extended to a more general form by Nernst.† These generalisations can be extended to the adsorption phenomena under consideration in the present communication.

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* 'Phil. Mag.,' 1908 [6], vol. 15, p. 499, and 1909 [6], vol. 17, p. 466, and 'Zeitsch. physikal. Chem.,' 1910, vol. 73, p. 129.

If an action takes place in a heterogeneous system, its principal seat will be at the limiting surfaces of the phases. In the case of the solution of a solid substance in water or of a solid base in an acid, the solvent action may be assumed to take place within an infinitely short interval of time. At any particular moment during the course of action, the solid phase will be surrounded by a layer of liquid of different composition to that of the remainder of the liquid phase. To establish equilibrium, molecules will pass through this layer (the diffusion phase), and the rate of action will be a function of the rate at which diffusion will take place. The constant A, representing the reaction rate, may be represented by the equation

$$A = \frac{\text{Area of surface} \times \text{coefficient of diffusion}}{\text{Thickness of the diffusion layer}}.$$

Now the coefficient of diffusion, and probably also the thickness of the diffusion layer, will depend upon another physical constant of the liquid phase, viz., the viscosity—the more viscous this phase, the more slowly the reaction will take place, other conditions being comparable.

These considerations will apply to the globulin system. Excess of globulin in the presence of salt solutions, if the hypothesis as to its nature be correct, forms a triphasic system containing the associated solid globulin aggregates (external phase), the dissociated globulin molecules in colloidal solution (internal phase), and the salt solution (dispersion medium). At the surface of the solid globulin the chief seat of action will be found, and all three phases will co-exist. Equilibrium will depend upon (*a*) the adsorption of the salt molecules by the dissociated globulin, (*b*) the rate of diffusion of these globulin molecules (with or without adsorbed salt molecules) from the solid globulin surface outwards, (*c*) the rate of diffusion of salt molecules inwards towards the same surface to establish equilibrium in the liquid phase, which has been altered in this position by adsorption of the salt from solution. The amount of adsorption is, as already stated, a function of the surface tension and concentration.* If, now, the rate of diffusion of the dissociated globulin molecules outwards from the limiting surface, and of the salt molecules inwards towards the same surface, be slow, the concentration of the globulin molecules at this point will be relatively high compared with that of the salt molecules, and a relatively small amount of adsorption will take place. As a consequence the dissociated molecules will tend to reaggregate to form the solid globulin. The reverse will hold if diffusion be rapid. If the conditions be such that a large amount of adsorption takes place, the solid globulin will completely disaggregate and a diphasic system will be formed; on dilution the amount of salt adsorbed will be diminished and reaggregation will take place.

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* ‘Zeitsch. Physik. Chem..’ 1897, vol. 23, p. 689.

† ‘Zeitsch. Physiol. Chem.,’ 1904, vol. 47, p. 52. See also Brunner, same volume, p. 56.

From these considerations it will follow *that the disaggregating action of salt solutions on the globulins is a function of two physical constants of the solutions, viz. the viscosities and the surface tensions. The higher the surface tensions and the viscosities, the smaller the disaggregating capacity.*

Quite similar considerations apply to the inhibitory action of salt solutions on the polymerisation or condensation of the methyleneimino-peptones. Here, again, three phases can co-exist, viz., the poly-product,† the simple methyleneimino-compound in colloidal solution, and the salt solution. If adsorption be sufficiently complete, the poly-product will not be formed, and the system will be diphasic. There is, however, a distinction between the system and the globulin system, which is probably more apparent than real. The precipitate formed by the addition of formaldehyde to Witte's peptone becomes, after a very short interval, insoluble, *i.e.* it will not dissolve in those salt solutions which inhibit its formation; it is, however, soluble in such solutions immediately after it is formed. Possibly a further chemical action, such as dehydration or scission of formaldehyde, takes place after polymerisation, with the formation of a product which can no longer be depolymerised or re-converted into the simple methyleneimino-product by water. The formation of such a product will tend to alter the equilibrium. It may be recalled that serum globulin also becomes insoluble in salt solutions after standing under water for some time, and possibly also undergoes a secondary chemical change of similar nature.

These conclusions have been confirmed by the quantitative investigation of the action of a series of salts on both the systems discussed above. The inorganic sodium salts—chloride, bromide and nitrate, iodide and sulphocyanide, mentioned in the order of decreasing surface tension—have a gradually increasing capacity for disaggregating globulins. Similar results were obtained with the sodium organic salts—lactate, acetate, monochloracetate, dichloracetate, trichloracetate, benzoate. It was found, however, that sodium iodide had a much greater disaggregating capacity than, for example, sodium monochloracetate, from which it differed very slightly in the surface tension as measured against air. The inorganic salt solutions, however, are considerably less viscous than those of the organic salts, and hence their disaggregating capacities are greater than those of the latter with the same surface tension. Sodium formate occupies an intermediate position. Its surface tension is somewhat less than that of sodium chloride, and its viscosity is appreciably greater; the latter constant is, however, smaller than those of the other organic salts. Sodium benzoate solution has nearly the same surface tension as that of the salicylate; it disaggregates less, owing to its considerably higher viscosity.

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* See adsorption equation given above.

† This term is used to indicate the more complex product.

In addition to the passive action in altering the physical properties of solutions, salts may also exert a disaggregating capacity in a more active manner. Hardy* and Mellanby† have shown that the salts of dyad metals (alkaline earths) have a greater solvent action on serum globulin than those of the monad metals; furthermore, salts of dibasic acids dissolve this substance more readily than those of monobasic acids. This action is probably due to the direct attraction of the salt molecules by the proteins, owing possibly to the difference of electric charge. These results have been corroborated. A similar action in the case of edestin is less marked.‡ The salts of the dibasic organic acids have a comparatively small solvent action on edestin; it is, however, greater than that of the salts of monobasic acids with solutions of high surface tension and viscosities. Analogies to these actions have been discovered in the investigation of salt action in other systems about to be described.

If the conceptions advanced above are correct they should apply also to systems other than those containing proteins. The experimental results which confirm them are given in a third communication.

The first of the simpler systems investigated was that containing phenol in the presence of salt solutions. As Rothmund has shown,§ if a mixture of the diphasic system phenol and water be heated, the phenol phase gradually takes up more water, and the aqueous phase takes up more phenol, until a point is reached at which the two phases assume the same chemical composition, and the system becomes monophasic. This point is known as the "critical solution temperature."

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* 'Journ. Physiol.,' 1905, vol. 33, p. 251.

† 'Journ. Physiol.,' 1905, vol. 33, p. 338.

‡ See Osborne and Harris, 'Amer. Journal of Physiology,' 1905, vol. 14, p. 151.

§ 'Zeitsch. Physikal. Chem.,' 1898, vol. 26, p. 433.

As it has been suggested that solutions of proteins and other hydrophil colloids are diphasic systems analogous to that of phenol and water, in which the amount of water in one of the phases can vary within wide limits, the investigation of the action of salts on this system was of some interest.

It was found that the addition of salts which lower the surface tension decreased the critical temperature of phenol and water. The critical solution temperatures, it is true, do not follow the surface tensions with strict accuracy; it must be remembered, however, that these constants have been measured at ordinary atmospheric temperatures only, whereas the critical solution points are relatively high. Nevertheless, the concordance is sufficiently striking. The viscosity in this case plays but little part, as there is passage of molecules to and fro from both systems. Furthermore, at higher temperatures, the difference of viscosity in different solutions tends to rapidly diminish.

The last series of systems investigated were those of crystalline substances in the presence of salt solutions. The action of salts in these cases was found to be analogous in nearly every respect to their action in systems containing proteins. The salt solutions with low surface tensions possessed a greater disaggregating capacity than those of the same series with higher surface tensions. The viscosity of the solutions also played the same part. In these systems, also, reaction must be considered as taking place at the surface of the two phases. Equilibrium will be attained when disaggregation takes place at the same rate as reaggregation. The lower the surface tension of the solution, the greater the tendency to disaggregate, and the less the tendency to reaggregate; the equilibrium point will be attained with a higher concentration of substance in salt solutions of low surface tension than in those of high surface tension. The more viscous the solution, the sooner will this equilibrium point be attained at the surface of the two phases (*i.e.* the smaller the quantity of solid substance which will have been dissolved), owing to the difficulty of the dissolved substances in diffusing from the solid phase.

These results were arrived at by determining the solubility of the five following substances in the same salt solutions as were employed in the investigations on protein systems—leucine, phenylalanine, caffeine, paratoluidine, and benzamide. The solution capacities of the inorganic salts and organic salts followed in the same order as their disaggregating action on the globulins. Salt solutions with low surface tension, such as sodium benzoate and sodium salicylate, dissolved appreciably more than those of high surface tensions. In the latter, the solubility of crystalline substances was generally less than that of water; in the former it was generally greater. These results are especially marked in the case of caffeine.

The solution capacities of the salts do not all follow in absolutely the same order in the case of all salts. The rate of diffusion and the surface tension at the junction of the phases could not be directly measured, and in some instances the former exerted a greater effect than the latter. Hence, in some cases, sodium formate has a greater solution capacity than sodium chloride, in others less. If, however, the salts be arranged in series, viz., the inorganic salts, with solutions of low viscosity, the organic salts, with solutions of high viscosity, with sodium formate forming an intermediate class by itself, it will be invariably found that the dissolving capacity of salt solutions of the same series bears a direct relationship to the surface tension.

It was also thought possible that the state of hydration of the salts in solution might exert some influence, in that, owing to combination of salt with water, less water in a normal solution would be available to act as solvent. W. Bilz* and H. C. Jones† have suggested that the abnormally great depression of freezing point of water produced by solutions of salts was due to the formation of hydrates in solution. The molecular depression of the freezing point of various salts was therefore investigated, and it was found that that of the organic salts was, as a rule, greater than that of the inorganic salts. Sodium formate, however, depressed the freezing point of water to about the same extent as did sodium chloride.

If the state of hydration played the chief *rôle* in determining the solubility of a crystalline substance in a salt solution, sodium formate solution should have had about the same solution capacity as sodium bromide and sodium nitrate. The solution capacity was invariably less, and less also in some cases than that of sodium chloride solution, which has a higher surface tension. It does not appear, therefore, as if the state of hydration plays a very direct part in determining the solution capacity of a salt solution, although it may play an indirect part, in that a highly hydrated salt may form a more viscous solution than one less hydrated in solution (compare in tables, viscosities and freezing points of sodium chloride and sodium iodide, sodium chloride and lithium chloride, sodium benzoate and sodium salicylate).

In the case of leucine and phenylalanine, both of which contain a carboxyl group, solutions of the salts of polyvalent metals possessed a greater solution capacity than those of the monovalent metals, the chlorides of the triad metal cerium dissolving more of these substances than any other solution investigated. This solution capacity was much less marked in the cases of caffeine (which acts only as a base in the presence of strong acids) and benzamide, and was entirely wanting in the case of the base para-toluidine. The solutions of salts of polybasic acids possessed, in the case of leucine and phenylalanine, a slightly higher dissolving capacity than did the salts of monobasic acids of corresponding surface tension and viscosity, and in this respect their behaviour was similar to that in the edestin system. Further investigation on this point is necessary, and also on the electrical charge carried by solid crystals.

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* 'Zeitsch. Physikal. Chem.,' 1902, vol. 40, p. 485.

† 'Publications of the Carnegie Institute,' 1907.

Minor differences in the solution capacity of salts of various metals were also noticed which could not be correlated with any physical property. Thus, for example, lithium salts possessed a slightly greater solvent capacity than did those of sodium and potassium (especially in the case of caffeine). Furthermore, strontium salts were intermediate in their action between those of calcium and barium, the calcium salts possessing a greater solvent capacity. These differences were often, however, only faintly marked.

Summary and Conclusions.

I. Complex substances which form colloidal solutions have the capacity of adsorbing other substances from those solutions, which, accumulating on their surface, interfere sterically with their chemical reactions. For this reason such colloidal substances do not in their reactions obey the ordinary laws of chemical mass action.

II. This conclusion has been illustrated by a study of the action of formaldehyde on Witte's peptone. Pauli's observations of the formation of protein salts of heavy metals can perhaps be explained in a similar way.

III. The analogy between the action of salt solutions in inhibiting chemical reaction of the complex proteins, and of their capacity to dissolve globulins, was noticed; the greater the inhibitory action, the greater the solvent power.

IV. These facts suggested an explanation of the chemical character of the globulins. These proteins are here regarded as substances of both markedly acid and markedly basic character, and capable of forming insoluble aggregates by the combination of the acid group of one molecule with the basic group of another; such aggregates will undergo a slight but definite hydrolysis in the presence of water, to form simpler dissociated globulin molecules. In the presence of salts, adsorption will take place on the surface of these simpler molecules, and thus prevent re-aggregation to the solid form and alter the hydrolysis equilibrium. The greater the adsorption of a salt, the greater, therefore, the solvent or disaggregating capacity of its solution for globulins.

V. The adsorption capacity of salts can be correlated with certain physical properties of their solutions. The higher the surface tension and the greater the viscosity, the smaller the adsorption and the disaggregating capacity. These conclusions have been verified experimentally; the influence of these physical properties on the action of salts can, however, be deduced from theoretical considerations, the influence of surface tension from the general study of adsorption phenomena, and that of viscosity by an extension of Nernst's generalisations on the rate of action in heterogeneous systems.

VI. The action of salts on systems other than those containing protein colloids is similar. Investigations have been made of the action of salts on the critical solution temperature of phenol and water, and on the solubility of the following crystalline substances :—*d.l.* leucine, *d.l.* phenylalanine, caffeine, benzamide, and *p.* toluidine.

VII. The critical solution temperature of phenol and salt solutions is a function of the surface tension of the latter.

VIII. The solubility of crystalline substances in salt solutions depends on the surface tensions and viscosities of the latter. In these systems the chief seat of action is at the limiting surfaces of the phases. Equilibrium may be regarded as established at the surfaces when dis-aggregation and re-aggregation are equal. The concentration at which this condition will exist is a function of the surface tension. The viscosity of the salt solution will affect the rate at which diffusion from the limiting surface takes place, and consequently the point at which equilibrium conditions are attained.

IX. The observations of previous observers as to the greater solubility of serum globulin in salts of the polyvalent metals, as compared with those of the univalent metals is confirmed. Similar phenomena were noticed in the case of leucine and phenylalanine. These phenomena were less marked in the case of edestin, and of caffeine and benzamide, and were entirely absent in the case of *p.* toluidine. It is suggested that they are due to the direct attraction of the salt molecules to the surfaces of the solid or disperse phases, possibly owing to the different electrical charges. This would alter the relative tensions of solutions at these surfaces as compared with the measurements of their surface tension against air. The serum globulin is also more soluble in salts of polybasic acids than in salts of monobasic acids. In the case of edestin and of the amino acids, these substances are perhaps very slightly more soluble in salts of dibasic acids than in those of monobasic acid of similar physical properties.* In other cases these phenomena were absent.

X. No evidence of the direct influence of the state of hydration of the salt in solution on the disaggregating capacity of the latter could be obtained.

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* An exact correlation of the solubility capacity with more than one physical constant is not possible with the data available.

The above generalisations as to the action of salts are probably capable of application to other systems. Considerable controversy has arisen as to the nature of the reaction between toxin and antitoxin. Arrhenius and his followers maintain, on the one hand, that it is analogous to the reaction between weak acid and weak base; other investigators hold, on the other hand, that it is of the nature of an adsorption phenomenon. If either or both of the reacting substances are of colloidal nature, adsorption of substances from the reaction medium (serum, etc.) would take place, and even if the reaction be of chemical nature, deviation from the ordinary laws of chemical mass action should be observed, owing to the sterical inhibition by adsorbed molecules. The amount of action would depend upon the medium in which it takes place. Investigations have been already commenced with the object of determining the influence of salts on the toxin-antitoxin reaction, and on other physiological and pathological phenomena.

B. EXPERIMENTAL.—I. THE PHYSICAL PROPERTIES OF SALT SOLUTIONS.

Throughout this work normal and not molar solutions have been employed. The sodium salts have been generally prepared by adding to 2N sodium hydroxide approximately the amount of acid necessary for neutralisation, one drop of a 1-per-cent. phenolphthalein being used as indicator. Neutralisation was completed by the addition of further quantities of acid in very weak solution, until the pink colour of the phenolphthalein just disappeared. The solutions after cooling were diluted till the normal concentration was attained.

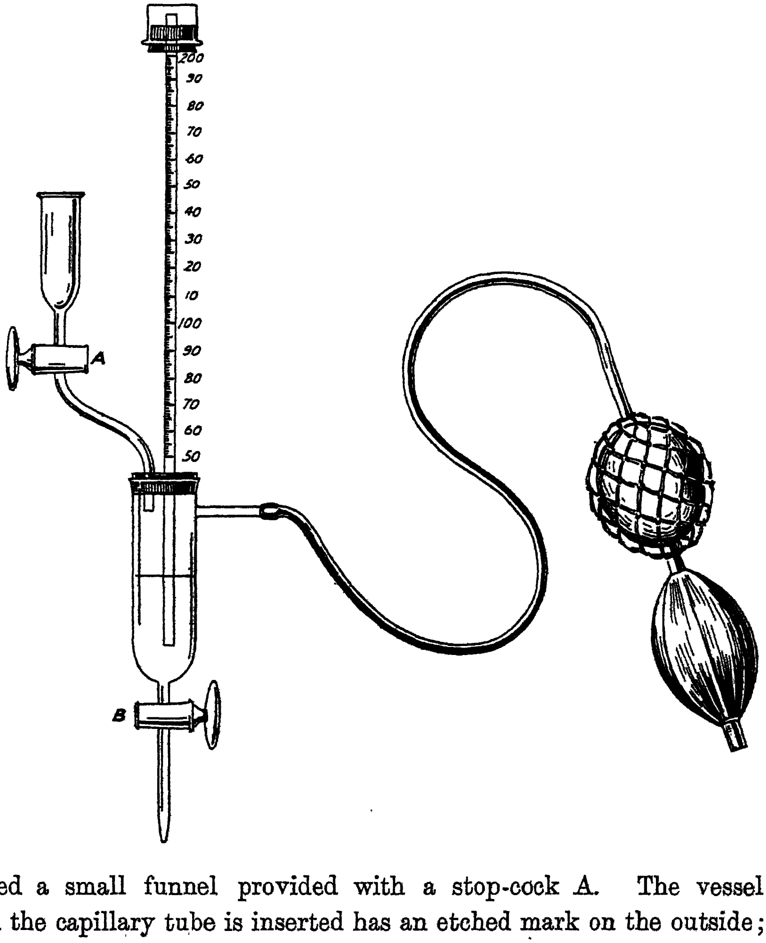
The solutions of chlorides of metals other than sodium were standardised by silver nitrate, volumetrically in the cases of magnesium and the alkali metals, gravimetrically in the case of the other alkaline earths. The sulphocyanide solution was standardised volumetrically by silver nitrate with the use of iron alum as indicator.

The sodium iodide solution was made up by neutralising 2N sodium hydroxide with hydriodic acid, which had been freshly distilled over red phosphorus, and was of only very faint yellow colour. The standard solution was kept in the dark.

Viscosity Determinations.

In order to work with small quantities of solution, a modification of Röntgen and Schneider's apparatus was employed, which is figured in the accompanying sketch.

A fine-bore capillary tube with opal-glass back graduated in the upper part from 50 to 200 mm., the lower mark of which is 50 mm. above an etched zero mark, is inserted through a rubber cork with the lower end downwards into a tube of 24 mm. diameter and 20 c.c. capacity, provided with a side-tube at the upper part near the cork, which is connected with an indiarubber ball, and drawn out at the lower end with a fine, almost capillary, tube, provided with a stop-cock B. Through the rubber cork is inserted a small funnel provided with a stop-cock A. The vessel into which the capillary tube is inserted has an etched mark on the outside; and, by means of a cathetometer, the capillary tube is so adjusted in the cork that its zero point is exactly opposite this etched mark. The liquid under examination is introduced through A, and by pressure of the rubber ball, and closure of the tap A, is caused to rise in the capillary. Stopcock A is then opened. Liquid is let out from the apparatus by stopcock B, or in by stopcock A, until the lower level is exactly at the zero point, which can be



easily recognised owing to the etched mark. When the liquid has come to rest, the height of the liquid in the capillary tube is read. The levels can be altered by squeezing the indiarubber ball, or by letting liquid out by tap B. The liquid should be passed through the apparatus and up the capillary tube several times until a constant reading is obtained. This is especially important in the case of liquids of high surface tension, which do not readily wet the glass. After using, the apparatus is washed out with water, and then with the fresh liquid of which the physical constant is to be measured. The little cup at the top of the capillary tube is for the purpose of collecting the wash-water and preventing it from soiling the outside.

Between every two or three determinations, a control measurement with pure water was interpolated, so as to ensure that there was no derangement of the zero point, and no error due to alteration of temperature.

All the solutions employed were made up with water which had been distilled in glass vessels over permanganate, and the apparatus and all the vessels employed had been previously soaked in concentrated sulphuric acid, and subsequently washed with the special distilled water before use.

The readings were made with a magnifying glass.

The surface tension determinations given in the accompanying table were the mean of several closely concordant readings (in most cases to within 0·1 mm.), many of them made after long intervals of time.

As unity was taken water, to 100 c.c. of which one drop of 0·5-per-cent. phenolphthalein in 50 per cent. alcohol had been added.* This addition made only a very minute difference in the reading. The specific gravities of the solutions were determined gravimetrically in the ordinary way.

Table I.—Surface Tensions of Normal Salt Solutions (Room Temperature).

Inorganic salts.		Organic salts.	
Sodium chloride	1·032	Sodium formate	1·020
„ bromide	1·024	„ lactate	1·013
„ nitrate	1·023	„ acetate	1·004
„ iodide	1·005	„ monochloracetate	1·002
„ sulphocyanide	1·000	„ dichloracetate	0·970
		„ trichloracetate	0·905
Lithium chloride	1·029	„ salicylate	0·902
Potassium „	1·028	„ benzoate	0·897
Magnesium „	1·030	„ malonate	1·0185
Calcium „	1·028	„ succinate	1·016
Strontium „	1·029	„ tartrate	1·011
Barium „	1·029	„ citrate	1·0105

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* See p. 108 for method of preparing standard solutions.

Viscosities of Salt Solutions.

The following measurements were made, as a direct comparison of the viscosities of the salts employed in the investigations was required under varying conditions of temperature, etc. The measurements were made at different temperatures also to determine whether any evidence as to the state of hydration of the salts in solution could be obtained. It was thought that the temperature coefficient would be large in the cases of heavily hydrated salts. The results indicate, however, that the state of hydration has probably only a minor influence on the viscosity. The low viscosity of sodium salicylate solution, compared with that of the benzoate, may possibly be due to the higher hydration of the latter (compare freezing-point determinations below). The results also indicate that sodium iodide and lithium chloride at lower temperatures may be more heavily hydrated than the other halides.

The determinations were made by means of Scarpa's apparatus,* in which, under a pressure somewhat higher than atmospheric, and indicated by means of a water manometer (in the results below, a plus pressure of 200 mm. of water was employed), a definite volume of liquid is made to pass both upwards and downwards through a capillary tube. In the results given below, the time required for passage in both directions is given. If attempts be made to transpose the numbers given below into absolute units by the equation $\eta = \kappa t$, where η = viscosity in absolute units, κ = apparatus-constant, and t = time of passage through capillary, it was found, using Thorpe and Rodger's values for water at different temperatures, that κ gradually became smaller with increasing temperature of the determination. This was due to the error arising from the formation of eddy currents, which were distinctly visible in the determinations made with liquids of low viscosity. As the error due to this factor is greater the lower the viscosity of the liquid, the differences between the determinations should be somewhat larger than those indicated in the table. The error does not detract, therefore, from the comparative value of the results.

The most salient features of the following table are the high viscosities of the organic monobasic sodium salts as compared with the inorganic salts. Sodium formate occupies an intermediate position with a viscosity lower than the highest inorganic salt viscosity (sodium iodide, which only has such a relatively high viscosity at quite a low temperature) but lower than that of any of the other organic salts. The high viscosity of the benzoate compared with that of the salicylate is also to be noted. No other features of the table call for special comment.

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* 'Archivio di Fisiologia,' 1905, vol. 2, p. 246.

Table II.—Relative Viscosities of various Salt Solutions (indicated by time (in seconds) of passage of a given quantity of fluid up and down a capillary tube).

Inorganic Salts.

Water.		Sodium chloride.		Sodium nitrate.		Sodium bromide.		Sodium iodide.		Sodium sulphocyanide.	
Temp.	Time.	Temp.	Time.	Temp.	Time.	Temp.	Time.	Temp.	Time.	Temp.	Time.
5·2	89·3	4·7	96·5	5·4	93·0	5·3	94·8	5·4	99·0	4·9	94·1
11·2	77·1	10·3	85·9	10·2	83·9	11·4	84·3	13·0	77·9	12·1	80·0
18·7	64·3	21·1	67·7	20·8	65·9	20·0	70·0	20·2	66·9	21·4	65·8
28·2	52·6	30·9	56·3	29·9	56·1	29·2	59·2	30·0	57·7	30·1	56·4
39·2	44·6	38·8	50·4	39·5	48·5	40·1	49·8	39·5	49·3	39·1	48·6
50·4	39·1	47·5	44·3	50·5	42·3	47·7	44·9	48·1	44·4	51·2	41·9
58·6	36·0	58·8	39·8	57·7	39·4	58·8	40·4	58·7	40·0	58·8	38·8

Lithium chloride.		Potassium chloride.		Magnesium chloride.		Calcium chloride.		Strontium chloride.		Barium chloride.	
Temp.	Time.	Temp.	Time.	Temp.	Time.	Temp.	Time.	Temp.	Time.	Temp.	Time.
5·6	101·3	4·7	85·6	3·4	113·7	4·0	105·4	4·7	102·8	4·3	101·2
11·7	85·4	12·0	74·0	10·9	92·7	11·0	88·2	11·9	88·8	11·7	85·2
21·0	69·8	19·9	64·5	20·0	74·2	20·1	71·0	20·7	71·7	20·5	70·1
30·4	58·1	30·0	52·4	30·6	60·5	28·8	60·3	38·9	52·7	31·8	57·0
40·2	49·8	39·4	46·1	40·2	51·7	39·4	56·9	57·5	43·3	40·0	50·7
49·5	44·1	48·4	40·9	50·0	45·5	51·3	43·3			48·5	45·2
58·9	40·1	58·8	37·2	59·8	40·9	59·4	39·7			58·0	40·7

Organic Salts.

Sodium formate.		Sodium lactate.		Sodium acetate.		Sodium monochloracetate.		Sodium dichloracetate.		Sodium trichloracetate.	
Temp.	Time.	Temp.	Time.	Temp.	Time.	Temp.	Time.	Temp.	Time.	Temp.	Time.
3·8	109·8	5·6	138·5	5·0	127·8	4·7	130·7	4·5	141·7	5·4	150·5
11·0	92·0	12·2	114·6	12·1	105·5	12·0	107·4	11·1	116·6	11·7	121·4
20·1	74·4	22·0	88·2	21·2	84·2	21·3	84·1	21·4	88·3	21·2	93·0
29·2	62·2	30·3	73·8	30·8	68·6	30·5	68·9	30·0	74·7	30·5	75·4
38·7	53·0	38·9	61·1	39·3	57·9	39·2	58·1	39·4	61·3	39·0	63·3
50·2	45·5	48·7	52·4	48·8	50·2	48·7	50·7	49·1	52·8	47·4	54·2
58·1	41·4	58·5	46·5	58·6	43·7	59·5	44·0	58·0	46·7	56·2	48·3

Table II—*continued.*

Sodium salicylate.		Sodium benzoate.		Sodium malonate.		Sodium succinate.		Sodium tartrate.		Sodium citrate.	
Temp.	Time.	Temp.	Time.	Temp.	Time.	Temp.	Time.	Temp.	Time.	Temp.	Time.
5·1	141·3	5·3	160·5	4·1	122·2	5·0	124·8	41·1	123·5	5·7	121·9
12·0	117·7	10·5	137·3	10·5	101·2	10·2	107·8	11·8	102·1	10·7	107·4
20·4	93·9	19·8	105·1	20·0	79·8	21·0	82·1	20·9	81·4	21·6	81·7
30·2	74·9	30·3	80·5	30·6	64·0	29·8	68·1	29·5	68·3	29·4	69·9
39·2	62·3	39·1	68·2	40·2	54·0	39·8	57·7	40·0	56·8	39·1	57·4
48·9	53·1	50·2	56·1	49·7	47·8	51·2	47·5	51·0	47·6	48·4	49·5
57·2	48·3	56·8	50·9	60·1	41·7	59·0	44·2	59·0	43·7	58·2	43·9

Molecular Depressions of Freezing Point.

These were determined by the ordinary Beckmann apparatus. The freezing point of normal solutions was directly determined.

Table III.—Molecular Depression of Freezing Points.

Salt.	Δ/M.	Salt.	Δ/M.
Sodium chloride	3·533	Sodium monochloracetate	4·008
„ bromide	3·725	„ dichloracetate	4·311
„ nitrate	3·212	„ trichloracetate	4·510
„ iodide	3·895	„ benzoate	4·095
„ formate	3·677	„ salicylate	3·375
„ lactate	3·940	Lithium chloride	3·851
„ acetate	4·000	Potassium „	3·225

No conclusions as to the *direct* influence of the state of hydration on disaggregating capacity can be drawn from the above tables. The great difference between the molecular depressions in the freezing point of benzoate and salicylate solutions may be noted. This may indicate differences in the state of hydration in solution, and thus differences in the viscosities; the degree of hydration may, therefore, possibly influence the disaggregating powers in an indirect way.

II. THE SOLUBILITY OF THE GLOBULINS.

Serum Globulin.

Previous estimations of the solubility of globulin in salt solutions have been made by Hardy* and Mellanby.† The general results indicate that serum globulin is more soluble in salts of polyvalent metals than in those of univalent metals, and in salts of polybasic acids than in those of dibasic acids. The results here appended amplify those of the observers just mentioned, in that they demonstrate the influence of surface tension and viscosity on the solvent property.

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* 'Journ. Physiol.,' vol. 33, *loc. cit.*

† 'Journ. Physiol.,' vol. 33, *loc. cit.*

The solvent (or disaggregating capacity) was determined by titrating 10 c.c. of a given suspension of the globulin with decinormal solution of the salt until the same solution grade was attained. The titration was carried out in a darkened room in a flat-sided trough, at the back of which was placed a constant source of light (electric lamp), the rays of which passed through a paper containing printed matter, held directly against the side of the trough nearest the light. The solution grade point arbitrarily chosen was that at which the printed matter was just legible through the liquid.

The serum globulin was prepared in the ordinary way from horse serum.

In Series I the globulin was purified by solution in ammonia, and precipitation from this solution by dilute acetic acid.

In Series II the sample was simply washed and the washings centrifuged off.

Table IV.—Amount of Decinormal Solutions necessary to add to Suspensions of Serum Globulin to Produce the same Solution Grade.

—	Series I. Strength of suspension 2·74 per cent.	Series II. Strength of suspension 1·79 per cent.
Sodium chloride	—	38·5
„ bromide	—	24·5
„ nitrate	—	22·8
„ iodide	—	16·0
„ sulphocyanide	—	15·5
„ formate	47·0	44·0
„ lactate	48·0	45·0
„ acetate	41·0	43·5
„ monochloracetate	33·0	34·0
„ dichloracetate	22·9	22·2
„ trichloracetate	17·0	14·7
„ benzoate	15·3	—
„ salicylate	12·3	11·25
„ malonate	—	17·5
„ succinate	—	21·0
„ tartrate	—	22·0
„ citrate	—	11·0

These results have been sufficiently discussed in the introduction.

Edestin.

A large number of determinations of the solubility of edestin in salt solutions have been carried out by Osborne and Harris.[*] The difference between the chloride, bromide, and nitrate and iodide are similar to the actions in the case of serum globulin. The results of Osborne and Harris have been extended by determining the solubility of edestin in a series of organic salts, and the results are indicated in the accompanying Table V.

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[*] 'Amer. Journ. Physiol.,' 1905, vol. 14, p. 151.

The edestin was prepared from hemp seed in the same way as that employed by Osborne and Harris, the final recrystallisation from sodium chloride having been carried out, after neutralisation of the solution, by allowing the latter to cool in an atmosphere free from carbon dioxide.

The experiments were carried out at ordinary room temperature in the same way as that described in Osborne and Harris' paper. Suspensions of 1 gramme in 10 c.c. of solution or of 2 c.c. in 20 c.c. solution were shaken for some time. When accurate quantitative determinations were made, the mixture was centrifuged, and the nitrogen determined in the supernatant liquid, which was pipetted off.

It was noticed that certain limits of concentration were reached between which the solubility of the edestin rapidly increased (compare the curves in Osborne and Harris' paper). At these limits the edestin became gelatinous on the addition of the salt solution. For these reasons the great differences of the solution capacities of the different salts could be readily demonstrated without the necessity of carrying out nitrogen estimations; in fact, when the edestin became gelatinous, accurate determinations of solubility were difficult.

Table V.—Solubility of Edestin in Sodium Salts of Organic Acids.

Numbers indicate grammes dissolved in 10 c.c. P. indicates that the Edestin forms a paste on addition of Salt.

Concentration salts.	Normal solution.	3N/10.	N/2.	N/4.	N/10.
Formate	Insol.	Insol.	Insol.	Insol.	Insol.
Lactate	”	”	”	”	”
Acetate	”	”	”	”	”
Monochloracetate	Forms paste	6·53	0·87	”	”
Dichloracetate	Very sol. P.	Very sol. P.	Very sol. P.	0·45	”
Trichloracetate	”	”	”	5·56	Traces only sol.
Benzoate	”	”	”	5·07	”
Salicylate	” Forms gel on addition of 3 gm. to 20 c.c.	Very sol.	Very sol.	Very sol.	1·46
Malonate	1·86	1·65	Traces only sol.	Insol.	Insol.
Succinate	0·65	0·29	”	”	”
Tartrate	1·94	0·78	”	”	”
Citrate	2·48	1·83	”	”	”

It will be noticed that the polybasic acid salts have only slightly greater solvent capacities for edestin than those of the monobasic acids. Furthermore, the influence of the valency of the metals is less marked. Interpolating from the curves of Osborne and Harris, it will be found that a 5-per-cent. solution of edestin can be formed under the conditions of experiment employed when the concentration is 5/6 normal. A similar strength edestin solution in sodium chloride is formed in the concentration of 13/20 normal. Bromide forms a 5-per-cent. solution in 9/20 normal (solubility capacity nearly equivalent to that of dichloracetate), whereas sodium iodide can form a solution of this strength in $\frac{1}{4}$ normal concentration, and has a solvent power equivalent approximately to those of the benzoate and salicylate. The higher solvent capacities of the solutions of inorganic salts are again well marked.

PART II.—*On the Action of Formaldehyde on Witte's Peptone.*

This portion of the communication contains the experimental details concerning the reaction of formaldelyde on Witte's peptone, the theory of which has been already discussed in some detail in the first part. The fraction which yields the precipitate with formaldelyde is that containing the more complex polypeptides, as the two following series of experiments show:—

Series I.—Fifty grammes of Witte's peptone were extracted, first with 600 c.c. of cold 80-per-cent. alcohol, and then with 400 c.c.. The alcoholic extract obtained was evaporated *in vacuo*, and the last traces of alcohol were driven off from the residue by taking it up in water and evaporating the solution thus obtained on a water bath. This formed Fraction I (*i.e.* part soluble in 80-per-cent. alcohol). The part of the peptone insoluble in 80-per-cent. alcohol was then extracted with 60-per-cent. alcohol, and the extract treated in a similar way to the 80-per-cent. alcoholic extract; this formed Fraction II. Fraction III was the part insoluble in 60-per-cent. alcohol. Each of the fractions was then made up to a definite volume in water, and the nitrogen in each was estimated. Portions were then taken from each fraction, and diluted so that solutions containing the same amount of nitrogen were obtained; 100 c.c. of each solution contained 0·2156 gramme nitrogen; 40 c.c. of these solutions were taken and to each was added 20 c.c. of 40-per-cent. formaldelyde. After standing, the precipitate was filtered off and nitrogen was estimated in aliquot parts of the filtrate.

In Fraction	I	the percentage of nitrogen	precipitated	was	8·3.
„	II	„	„	„	15·6.
„	III	„	„	„	40·0.

Another fraction with the same nitrogen content was prepared from a solution of peptone which had been dialysed for three or four days against running water. Of this preparation, 23·3 per cent. was precipitable by formaldehyde.

Series II.—Fifty grammes of Witte's peptone were extracted successively with two portions of 500 c.c. hot 70-per-cent. alcohol. The insoluble portion (Fraction III) was then washed with cold 70-per-cent. alcohol, and finally with absolute alcohol, and then dried on a water bath. It weighed, when dry, 13·5 grammes.

From the hot aqueous alcohol a second fraction (Fraction II) separated on cooling; this was washed with 70-per-cent. alcohol, then with absolute alcohol and then dried. Fraction I was the portion soluble in 70-per-cent. alcohol.

These were also dissolved up, and solutions made containing the same amount of nitrogen (0·8848 gramme per 100 c.c.).

To determine the complexity, 10 c.c. of each fraction was treated with 5 c.c. formaldehyde and then titrated with N/10 sodium hydroxide by Sörensen's method. The following were the quantities of alkali necessary to neutralise to phenolphthalein :—

Fraction	I	8·9 c.c.
„	II	7·0 „
„	III	5·2 „

To 10 c.c. of each fraction was added 5 c.c. of formaldehyde.

Fraction I was at first clear, and became turbid after standing.
„ II gave an immediate precipitate.
„ III gave a strong clot almost immediately.

After standing for 16 hours the precipitates were filtered off and the nitrogen was estimated in 10 c.c. of the filtrate.

The following were the percentages of nitrogen precipitated by formaldehyde :—

In Fraction	I	25·3 per cent.
„	II	55·7 „
„	III	68·6 „

These results indicate that the larger part of the formaldehyde precipitate is due to the more complex polypeptides contained in the peptone.

Inhibitory Action of Salts.

If minute quantities of caustic alkali be added to the peptone solution, no precipitate will form on the addition of formaldehyde. The formation takes place, however, after the addition of acids; this addition must not, however, exceed that necessary to produce a turbidity in the peptone solution (precipitation of nucleo-protein). The precipitation is also inhibited by the sodium salts of weak acids. This is due to the fact that the solution, after the addition of aldehydes, contains acids of the type CH : N·R·COOH, which are sufficiently strong to decompose the sodium salts of weak acids. For this reason, salts of weak acids, such as sodium acetate and sodium propionate, do not fall in the same relative position with regard to their inhibitory action as would be expected from the theory connecting viscosity and surface tension with this function. This fact may serve to explain other minor deviations shown in the accompanying table.

In these experiments, of which the results are here recorded, 5 c.c. of a Witte peptone solution were diluted to 10 c.c. with varying quantities of normal solutions of different salts, so that a series of solutions of 2½ per cent. peptone in concentrations varying from N/10 to N/2 of the different salts was obtained. To each dilution was added 2·5 c.c. of a 35-per-cent. formaldehyde solution. The degree of precipitate formation at varying intervals was then noted.

Table giving Inhibitory Action of Various Salts on the Formation of Poly-methyleneimino-peptone.

0 indicates	no precipitate.	3 indicates	marked opalescence.
1 „	faint turbidity.	4 „	quite opaque mixture.
2 „	opalescence.	5 „	precipitate formation.

The three numbers under each heading indicate the observations after respectively 2½, 4½, and 20 hours.

Concentrations.	$\frac{5N}{10}$.			$\frac{4N}{10}$.			$\frac{3N}{10}$.			$\frac{2N}{10}$.			$\frac{N}{10}$.		
Sodium chloride	3	3+	5	3	+3	5	4	4	5	5	5	5	5	5	5
„ bromide	0	0	0	0	0	0	−1	−1	+3	4	+4	5	5	5	5
„ nitrate	0	0	0	0	0	0	−1	−1	+3	+3	4	5	5	5	5
„ iodide	0	0	0	0	0	0	0	0	0	+3	4	4	5	5	5
„ sulphocyanide	0	0	0	0	0	1	2	2	3	4	4	5	5	5	5
„ formate	0	0	0	−1	−1	1	3	3	3	5	5	5		—	
„ lactate	+1	+1	+1	2	2	2	4	4	4	5	5			—	
„ acetate	0	0	0	0	0	0	0	0	−1	4	4	+4		—	
„ monochloracetate	0	0	−1	2	2	3	3	+3	5	5	5	5	5	5	5
„ dichloracetate	0	0	0	0	0	1	+2	3	5	5	5	5	5	5	5
„ trichloracetate		—		0	0	0	1	1	1	4	4	5	5	5	5
„ benzoate		—		0	0	0	0	0	0	0	0	0	4	4	4
„ salicylate		—		0	0	0	0	0	0	1	1	1	5	5	5
„ malonate		—			—		0	0	0	0	0	0	0	0	0
„ succinate		—			—			—			—		0	0	0
„ tartrate		—		0	0	0	−1	−1	−1	4	4	4	5	5	5
„ citrate		—			—		0	0	0	0	0	0	0	0	0

The general parallelism between these actions and that of the solvent action on serum globulin is manifest, although there are in case of weaker acids noticeable deviations, for the reasons already described.

The salts of polyvalent metals have also a slightly stronger inhibitory action than those of the univalent metals, and this is indicated in the following tables. The experiments here recorded were carried out by adding 10 c.c. of the normal salt solution to 10 c.c. of 5-per-cent. peptone solution, and then precipitating by 4 c.c. of 40-per-cent. aldehyde solution. After standing for a day the precipitate was filtered off, and the nitrogen was estimated in aliquot parts of the filtrate. The total nitrogen was estimated also in a corresponding portion in which no precipitation took place, and in a portion in which 10 c.c. of peptone was diluted with 10 c.c. of water instead of salt solution.

Table giving the Relative Amount of Precipitation in Presence of Various Chlorides. 2½ per cent. peptone in N/2 solutions of salts.

	Chlorides.		Chlorides.
Water	100·0	Magnesium	25·5
Lithium	49·0	Calcium	4·5
Sodium	49·5	Strontium	24·5
Potassium	40·0	Barium	26·0

Part III.—*On the Solubility of Phenol and certain Crystalline Substances in Salt Solutions.*

The theory and discussion of the results recorded in this part have been already dealt with in some detail in Part I.

Critical Solution Temperature of Phenol and Salt Solutions.

The compositions of the two phases, phenol and water, were investigated in detail many years ago by Rothmund.* It may be recalled that if a mixture of the two substances be heated, the phenol phase becomes richer in water, and the aqueous phase richer in phenol until a point is reached when the system becomes monophasic. The temperature at which this will happen will depend upon the readiness with which disaggregation takes place, and should consequently be a function of the surface tension at the limiting surfaces of the phases. The lower the surface tension of the salt solution therefore, the lower should be the critical solution temperature. This hypothesis is fully borne out by the results recorded in the accompanying table.

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* 'Zeitsch. Physikal. Chem.,' 1898, vol. 26 p. 433.

Fifteen grammes of crystalline phenol were mixed with 25 c.c. of N/10 salt solutions in a wide-mouthed test-tube provided with a stirrer, and with a thermometer inserted into the mixture. This was inserted into a litre beaker containing water, which was also provided with a stirrer, and which was slowly heated on a sand bath; both the mixture in the test-tube and the water were continually stirred the whole time. As critical solution temperature is arbitrarily chosen that point at which the phenol-salt solution mixture is converted from an opaque to an opalescent fluid, a process which takes place within the interval of one-tenth of a degree. On further heating the opalescent fluid gradually becomes clear; on cooling again, with continual stirring, opalescence and final opacity reappear, and the point at which the opalescent fluid becomes opaque is only a fraction of a degree lower than the point observed when the change took place in the reverse direction.

With normal solutions of the salts of lower surface tensions, such as sodium trichloracetate, the system is monophasic even at quite low temperatures (4° C.).

Table I.—Critical Solution Temperatures of Phenol and N/10 Salt Solutions.

Solutions.	Temperature.	Solutions.	Temperature.
Sodium chloride	74·3	Sodium formate	70·0
„ bromide	73·7	„ lactate	68·5
„ nitrate	71·8	„ acetate	66·9
„ iodide	72·1	„ monochloracetate	66·1
„ sulphocyanide	68·2	„ dichloracetate	63·1
		„ trichloracetate	58·0
Lithium chloride	74·3	„ benzoate	49·1
Potassium „	73·2	„ salicylate	49·3
Magnesium „	75·2	„ malonate	77·1
Calcium „	75·0	„ succinate	74·8
Strontium „	74·9	„ tartrate	77·1
Barium „	75·0	„ citrate	77·0
		„ chloride	74·3

Solubility of Crystalline Substances in Salt Solutions.

The benzamide, caffeine, and *p*-toluidine were Kahlbaum's preparations.

The phenylalanine was prepared synthetically by the method of E. Fischer.*

The *d.l.* leucine was prepared directly from commercial isoamyl alcohol. From the latter, isovaleraldehyde was prepared by the method of Bouveault and Rouszet† by dropping the alcohol into a warmed bichromate mixture and allowing the aldehyde to distil off as soon as it is formed. The latter, after separating from the aqueous layer, was dried over fused sodium acetate, and then fractionated with a Young rod and disc still-head and the fraction boiling below 120° was separately collected. This was converted into the bisulphite compound, which was washed with ether, and air-dried, decomposed with a small excess of bicarbonate and the aldehyde then distilled off. To the aldehyde was then added, little by little, and with constant cooling, half the volume of ordinary concentrated ammonia, and the mixture, after standing a day, completely solidified. The valeraldehyde ammonia compound was then washed with ether, and air-dried. The compound from 300 grammes aldehyde was then treated in three portions with 300 c.c. water, and to the suspension was added 150 c.c. anhydrous hydrocyanic acid, diluted to 270 c.c. with water. The cyanammonia compound was then hydrolysed, and the remainder of the preparation completed by the method of E. Fischer.* From 300 grammes of aldehyde were obtained 200 grammes of twice recrystallised pure inactive leucine.

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* 'Ber.,' 1904, vol. 37, p. 3062.

† 'Bulletin de la Soc. Chim.,' [3], vol. 11, p. 301.

The determinations of solubility were carried out by mixing 1 gramme (or more if 1 gramme were insufficient for complete saturation) of the crystalline substance with 30 c.c. of the salt solution in short wide-mouthed test-tubes, which were then tightly corked, and clamped on to a rotating axis in a thermostat, and thus, by means of a motor, kept in a state of constant motion, generally for a period of 36 hours. At the end of this time the liquid was filtered from the undissolved solid by means of a water pump through a perforated platinum plate covered with asbestos, into a flask with a narrow neck graduated in tenths of a cubic centimetre between 20 and 30 c.c., which was carefully calibrated before use, and which was provided with a side-tube near the mouth of the flask, turned upwards so as to connect with the water-pump for suction. The whole apparatus was immersed in the thermostat during filtration, and the amount of liquid filtered was directly read.

The nitrogen was determined in this liquid by means of Kjeldahl's method, except in the case of the nitrates and sulphocyanides. In both these cases the leucine and phenylalanine were estimated by Sörensen's formaldehyde titration method, and in determinations of the solubility of other substances in sulphocyanide the undissolved crystallised substance left on the filter-plate was dissolved in some organic solvent, and the nitrogen determined in this solution after filtering through blotting-paper. In the latter case there is a slight error, owing to the fact that it is impossible to wash the crystals. The Kjeldahl method of estimation was, in the case of phenylalanine and leucine, preferred to the Sörensen method, as the end point of titration is a certain pink colour, and not the first appearance of pink. Nevertheless, a series of solubility determinations carried out by this method in the case of leucine agreed well with the results obtained by the Kjeldahl method.

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* 'Ber.,' 1900, vol. 33, p. 2370.

The ammonia was distilled off through hard-glass well-cooled condensers, to avoid the action of the hot liquid on the glass. Sodium alizarine sulphonate was the indicator used for titration, and the ammonia present in the various reagents as an impurity was estimated and the necessary corrections made. It was only found in the sulphuric acid and lithium chloride, and the correction in each case was only a fraction of a cubic centimetre.

Table II.—Solubility of Leucine and Phenylalanine. Temperature $23^{\circ}\cdot7 \pm 0^{\circ}\cdot1$ C.

	Leucine.			Phenylalanine.		
	A. Mg. mol. per litre.	B. Grammes per litre.	C. Relative solubility, $H_2O = 100$.	A. Mg. mol. per litre.	B. Grammes per litre.	C. Relative solubility. $H_2O = 100$.
Water	73·1	9·57	100·0	85·2	14·06	100·0
Sodium chloride	63·9	8·37	87·4	72·6	11·98	85·2
" bromide	68·9	9·03	94·3	83·2	13·77	97·7
" nitrate	68·9	9·03	94·3	86·6	14·29	101·6
" iodide	72·7	9·52	99·4	93·4	15·41	109·6
" sulphocyanide	78·2	10·24	106·9	99·9	16·48	117·3
" formate	65·1	8·53	89·0	75·7	12·49	88·8
" lactate	53·9	7·06	73·4	58·6	9·67	68·8
" acetate	57·6	7·55	78·8	66·9	11·04	78·5
" monochloracetate	61·4	8·04	84·0	74·6	12·31	87·5
" dichloracetate	62·5	8·19	85·5	80·0	13·20	93·9
" trichloracetate	66·2	8·67	90·6	86·6	14·29	101·6
" benzoate	68·3	8·94	93·4	96·1	15·86	112·8
" salicylate	81·3	10·65	111·2	120·8	19·93	141·7
" sulphate	59·3	7·77	81·1	69·4	11·45	81·4
" malonate	60·0	7·86	82·1	70·6	11·65	82·8
" succinate	57·7	7·56	78·9	69·1	11·40	81·1
" tartrate	57·2	7·49	78·2	65·2	10·96	76·5
" citrate	61·2	8·02	83·7	72·6	11·99	85·3
" chloride	63·9	8·37	87·4	72·6	11·98	85·2
Lithium "	69·2	9·06	94·6	81·4	13·53	95·5
Potassium "	65·4	8·57	89·5	77·5	12·79	90·9
Magnesium "	81·6	10·69	111·6	94·3	15·56	110·6
Calcium "	87·4	11·49	119·5	106·2	17·52	124·6
Strontium "	83·0	10·87	113·5	96·5	15·92	113·3
Barium "	78·8	10·32	107·8	97·8	16·14	114·7
Cerium "	164·6	21·56	225·1	175·0	28·87	205·4

The results are recorded in three columns. In Column A the solubility in milligramme molecules per litre is given; in Column B the solubility in grammes per litre of solvent; and in Column C the relative solubilities, that in water being taken as 100.

Table III.—Solubilities of Caffeine, Benzamide, *p*-toluidine. Temperature 23°·7 ± 0°·1 C.

	Caffeine.			Benzamide.			*p*-toluidine.		
	A.	B.	C.	A.	B.	C.	A.	B.	C.
Water	98·5	19·109	100·0	105·6	12·78	100·0	66·6	7·126	100·0
Sodium chloride	65·7	12·75	66·7	80·5	9·74	76·2	50·3	5·38	75·5
,, bromide	105·3	20·43	106·9	89·9	10·88	85·2	56·0	5·99	84·5
,, iodide	225·6	43·77	229·0	105·2	12·73	99·9	68·4	7·32	102·7
,, sulphocyanide	288·0	55·87	292·4	112·2	13·57	106·2	135·0	14·44	202·7
,, formate	56·7	11·00	57·5	79·7	9·64	75·4	42·7	4·57	64·1
,, lactate	46·9	9·10	47·6	77·5	9·38	73·3	36·8	3·93	55·2
,, acetate	48·9	9·49	49·9	80·0	9·68	75·7	41·4	4·43	62·1
,, monochloracetate	84·9	16·47	86·2	101·9	12·33	96·5	reaction	—	—
,, dichloracetate	167·1	32·34	169·6	109·2	13·21	103·4	66·4	7·10	100·0
,, trichloracetata	416·6	80·82	422·9	137·9	16·69	130·5	71·7	7·67	107·6
,, benzoate	776·3	150·60	787·8	164·9	19·95	156·1	81·7	8·74	122·6
,, salicylate	1188·0	230·47	1206·1	244·7	20·61	231·7	118·9	12·72	178·5
,, sulphate	49·4	9·58	50·1	67·9	8·21	64·3	38·4	4·11	57·7
,, malonate	42·3	8·20	42·9	70·2	8·49	66·4	29·2	3·12	43·8
,, succinate	44·1	8·55	44·7	72·0	8·71	68·1	37·4	4·00	56·1
,, tartrate	44·1	8·55	44·7	68·6	8·30	64·9	34·1	3·65	51·0
,, citrate	41·0	7·95	41·6	69·5	8·41	65·8	31·5	3·37	47·3
,, chloride	65·7	12·75	66·7	80·5	9·74	76·2	50·3	5·38	75·5
Lithium ,,	110·2	21·80	114·0	89·2	10·69	83·5	56·9	6·08	85·9
Potassium ,,	68·0	13·19	69·0	83·7	10·13	79·2	52·6	5·63	78·9
Magnesium ,,	83·6	16·22	84·8	90·1	10·90	85·3	53·1	5·68	79·7
Calcium ,,	84·6	16·41	85·9	93·4	11·26	88·4	51·5	5·51	77·3
Strontium ,,	79·2	15·36	80·4	91·0	11·01	86·1	53·2	5·69	79·9
Barium ,,	76·6	14·86	77·7	89·2	10·79	84·4	47·7	5·10	71·7

In the above table the salts are divided into five subdivisions, viz., the inorganic monobasic salts, or salts of low viscosity, sodium formate, a salt with viscosity between those of the inorganic salts and the other organic salts, the organic monobasic salts with high viscosities, the salts of polybasic acids, and salts of metals other than sodium. The monobasic sodium salts are placed in descending order of their surface tension in the columns.

This arrangement indicates at a glance the generalisations arrived at in the theoretical introduction to the three papers.

On a Remarkable Pharetronid Sponge from Christmas Island.

By R. KIRKPATRICK.

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(PLATES 10 AND 11.)

The foundation of our knowledge of the Pharetron sponges was laid by Zittel in 1879 (9, p. 11). He found, when classifying the fossil sponges, that after the Dictyonines, Lithistids, Tetractinellids, and Monaxonellids had been sorted out, there still remained numerous specimens, mainly calcareous, and ranging from the Devonian to the Cretaceous formations, in which the skeleton was formed of a network of anastomosing calcareous fibres with a very finely fibrous structure.

In 1872 Haeckel (3A, vol. 1, p. 341) had declared that no fossil sponges belonging to the order of Calcisponges had been found, nor were ever likely to be, judging from the delicate texture of known Calcisponges.

At first Zittel regarded the fossil "Calcispongia fibrosa" or "Fibrous sponges" as members of a wholly extinct order, in no way related to that of the true Calcispongiæ. Some regarded the fibrous sponges as fossil horny sponges, others as altered siliceous sponges, others again as corals. Zittel found, however, in the course of his investigations, that the fibrous markings in the fibres were due to the presence of uniaxial or three- or four-rayed spicules. This discovery led him to regard the fibrous sponges from a wholly new point of view, viz., as true Calcareous Sponges.

As the fossil Calcarea known at that time were, with one exception, apparently very different from the recent sponges belonging to Haeckel's three families (Ascones, Sycones, Leucones), and as they seemed to be a more or less homogeneous group, Zittel included them in a new family, the Pharetrones.* His conclusions were at first disputed, and it was thought that the spicules composing the fibres were originally siliceous bodies, which had undergone calcification in the course of becoming fossilised.

In 1882 Hinde (4, p. 185) described several very well preserved Pharetrones, in which not only were the spicules visible in the fibres, but also separate three- and four-rayed spicules and "tuning-forks" could be distinguished in a layer on the surface of the sponge. All doubts as to Zittel's discovery were now set at rest. Neither Zittel nor Hinde attempted to arrange the numerous genera of Pharetrones into sub-groups, but described the external form, the canal system, the size, shape, and structure of the fibres, and, wherever possible, the spicules composing them.

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* φαρέτρα, a quiver, from the fancied resemblance of the Pharetron fibre to a quiverful of arrows.

The spicules which make up the so-called "Pharetron fibre" are closely packed, and arranged more or less parallel to each other and to the long axis of the fibre. The spicules may be rod-shaped, or nearly linear three-rayed forms, or large centrally situated three- or four-rayed forms surrounded by slender rods or triradiates.

The composition of the Pharetron fibre has been the subject of much discussion. Dunikowski (3, p. 299) and Rauff (7, p. 204) considered that the spicules were, in the living sponge, simply in apposition and not cemented together, and that the present fused vitreous condition of the fibres has resulted from fossilisation. Steinmann (8, p. 111), on the other hand, considers that the spicules were joined together by a cement formed by the living sponge. Dunikowski regarded the Pharetrones simply as a sub-family of Haeckel's family of Leucones. Rauff, who thought that Dunikowski had gone too far in so doing, retained the family Pharetronidæ, on account of the spicules being arranged into fibres, the spicules of the Leucones not being so arranged.

In 1892 (1, p. 143) and 1898 (2, p. 15) Döderlein described a remarkable recent stony calcareous sponge (*Petrostroma schulzei*) from Japan. The main skeleton was constructed of large four-rayed spicules cemented together so as to form a firm solid meshwork, the spicules in the older parts being more or less completely surrounded with calcareous cement; also there was a dermal layer of loose three- and four-rayed spicules and separate small bundles or "fibres" of "tuning-forks." Döderlein placed *Petrostroma* in a new group, the Lithones.

In 1893 Rauff (7, p. 204) classified all the Calcarea, fossil and recent, into two orders, Dialytina, with separate spicules, and Lithonina (including only *Petrostroma schulzei*, Död.), with spicules joined by zygosis into a solid framework (like the desmas of Lithistida). It was not known at the time that the spicules of *Petrostroma* were joined together by cement, as was explained by Döderlein (2, p. 15) in his second communication on that genus. Rauff placed the Pharetronidæ next to the Leuconidæ.

Whether Rauff's division of Calcarea into two groups, viz., one with separate spicules and one with zygosed spicules, could have been retained or not, it is now needless to enquire; but it could hardly be maintained that the Calcarea should be divided into two orders, viz., one without cemented spicules, and the other with cemented spicules. Among siliceous sponges the mere presence or absence of cement (spongin) is not of much importance. In *Halichondria* we may find separate scattered spicules, in *Reniera* they are often joined into a network cemented at the nodes with spongin, the strands being formed either of one spicule or of a bundle; in *Chalina*, the bundles of spicules are wholly enveloped in spongin, and in certain horny sponges derived from Chalininæ the spicules have gone, and a network of spongin fibres alone remains. So in Calcarea, the spicules are separate and irregularly arranged in the parenchyma in *Leucandra;* in *Plectroninia* the spicules of the main skeleton are joined chiefly at the ends; in *Petrostroma* and *Minchinella* the calcareous cement in the older parts of the sponge completely envelopes the spicules, and in the sponge described below a network of cement alone remains.

In 1900 Minchin (6, p. 110) included the Pharetronidæ as one of the five families of the Heterocoela, characterised by the possession of tuning-fork spicules; and divided it into two sub-families, viz., (i) Dialytinæ, Rauff, with uncemented spicules, (ii) Lithoninæ, Döderlein, with body-spicules united by cement into a rigid framework.

Doubtless some of the genera placed among the Dialytinæ will be found to belong to the Lithoninæ, and possibly others to the Sycettidæ and Grantidæ of Dendy.

Steinmann's classification (8, p. 112) is nearly the same as Minchin's; for he divides the Pharetrones into (i) Lithonina, and (ii) a group including Inozoa (Dialytina pars), massive sponges with leuconoid canal-system, and Sphinctozoa, moniliform sponges with syconoid canal-system; but Steinmann could not employ the term Dialytinæ, for he regards the Pharetron fibre as a bundle of cemented spicules.

The classification used in this paper is that of Minchin, with an additional new sub-family. The specimens about to be described were obtained by Dr. C. W. Andrews during his second visit to Christmas Island. On one occasion, the steamer which plies between Singapore and the island got its anchor fixed among the rocks in 46 fathoms off the shore of the latter place. After tugging for a long time with full steam on, the ship at last got free. The anchor brought away a mass of rock on which were found four specimens of the new sponge. The specimens were detached from the rock a little time after capture, and put into methylated spirit.

The sponges were found to belong to a new genus and species of Pharetronidæ, which family now includes six recent species. The new species will certainly prove to be of very great interest both to the student of recent sponges and to the palæontologist, for not only is the rigid main skeletal network entirely devoid of spicules, but the surface of the sponge is covered with a dermal armour of large calcareous scales.

I propose to name the genus and species *Murrayona** *phanolepis*,† and to place them in a new sub-family Murrayoninæ below and next to the Lithoninæ.

Description of Murrayona phanolepis, *gen. et sp. nov.*

The specimens are in the form of small pear-shaped or massive stony nodules, either sessile or with a very short thick stem, and are attached by a flat expanded base to the rock. Two of the specimens are complete (Plate 10, figs. 3, 3A), and the other two (Plate 10, figs. 1, 2) macerated out and devoid of both a dermal layer and of soft tissues. The largest is about the size of a small Barcelona nut (Plate 10, fig. 2).

The best preserved specimen, A, has been kept entire, and the second complete specimen used up for sections of soft tissues and skeleton. Specimen A (Plate 10, figs. 3, 3A, 3B) is in the form of a minute, pear-shaped nodule, 8 mm. high and 7 mm. broad, with a very short thick stem 1 mm. high and 2 mm. in diameter, expanding below into a flattened disc of attachment. The colour is pale yellow, and the surface glistening. Under a lens an areolated pattern, formed by imbricating scales, can be seen.

A little to one side of the summit is a small oscule about 0·4 mm. in diameter, and above the middle of the opposite aspect is an equatorial groove (figs. 3A, 3B) extending a little more than half-way round the sponge. Besides the oscule and pores of this poral groove there are no other openings into the sponge, the rest of the surface being covered with an impenetrable coat of scales (fig. 3B).

The edges of the scales are free, that is to say there is no continuous membrane passing from edge to edge. Beneath the semi-translucent layer of scales can be seen, under a low power, the more or less parallel edges of the surface-lamellæ of the main skeletal framework, which form supporting beams to the roof of scales; and here and there between the scales and main skeleton very minute triradiate and tuning-fork spicules are visible. The poral groove shows a network of small meshes formed by bundles of triradiates.

The *canal-system* belongs to the leuconoid type. The pores are limited to a groove situated on one side of the sponge about midway between the summit and base (Plate 10, fig. 3B). The groove, which is about 8 mm. long,

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* In honour of Sir John Murray, K.C.B., F.R.S., who has made a very generous donation towards the expenses of a small dredging expedition to Christmas Island.

† φανός, bright, glistening; λεπίς, scale.

1 mm. deep, and 2 mm. broad, is divided into two nearly equal parts by a narrow vertical band of scales. The rounded or polygonal meshes of the network of triradiates are about 0·2 mm. in diameter, and one pore is contained in each mesh (Plate 10, fig. 8).

The pores lead into sub-dermal spaces, which either abut directly on to masses of flagellated chambers filling in the surface-meshes of the main skeletal network just below the pore-area (Plate 10, fig. 5), or are continued into canals passing into the sponge.

The flagellated chambers are oval or spheroidal, and of large size, being about $65 \times 50\,\mu$ in long and short diameters. Minute prosopyles were visible in some instances, also a circular apopyle, about 20 μ in diameter.

The chambers line the walls of tubular anastomosing canals, occupying the spaces of the main skeleton, just as in *Minchinella lamellosa*, Kirkp. (Plate 11, fig. 13). The exhalant canals pass up into the floor and walls of the single terminal exhalant canal or cloaca, which opens by the oscule. The cloaca is from 1 to 2 mm. deep.

The structure of the collar-cells and of the other soft tissues was not to be made out clearly, owing to the sponges not having been properly fixed at the moment of capture, and as there is some prospect of getting fresh material suitably preserved, no attempt has been made to describe the cell-structure.

The Skeleton.

The skeleton will be described under three heads, viz.: (1) the main body-skeleton; (2) the poral and sub-dermal spicules; and (3) the dermal scales.

The *main skeletal framework* has a structure wholly unique in sponges. It is constructed of a strong network of solid calcareous fibres, entirely devoid of spicules, either in the axis of the fibres or on the surfaces of the same. The strands of this remarkable network vary greatly in shape and size, but they are mostly laminate, *i.e.* they are thin in proportion to their breadth. An average-sized strand measured 375 μ in length, 150 μ in breadth, and 45 μ in thickness, and the oval mesh which it bounded was 375 μ long and 150 μ broad; but the meshes and strands may be considerably larger, viz., up to 500 μ long. Usually, the meshes are longer in the vertical plane of the sponge than in the horizontal (Plate 10, figs. 4, 7, 9).

At the surface of the body the skeleton tends to form longitudinal flanges with free edges which slightly overlap, and which support the dermal skeleton.

The strands show a laminate construction, as if they had been laid down in layer upon layer of flakes, and here and there sutural lines can be seen between masses of flakes. Even under a low power, the microscopic structure is seen to be coarsely fibrillar (Plate 11, fig. 16), the fibrillæ radiating usually in a fan-like manner. Sometimes the flakes are circular or long oval in shape, with the fibrillæ radiating out all round, and more or less in one plane. The fibrillæ under a high power show conical projecting ends, and are separate and distinct for a little distance below the surface (Plate 11, figs. 14, 15).

In two places in the ground-down sections of a whole specimen, an axial spicule appeared to be present in the strands, but under higher powers the appearance was seen to be due to interior flakes of elongated shape with the fibrillæ arranged on each side of a smooth central raphé. If I had found merely some relic of a spicule—but I failed to do so after a most careful search—I would still have regarded the skeleton as a new type of construction.

The *spicules* are all triradiate, but of different shapes, viz.:—

(*a*) Equiangular, equal-rayed or nearly equal-rayed, pyramidal, that is, with the ends resting on a plane different from the meeting-point of the rays. The rays are 55 μ long, and 10 μ thick at the base, with blunt or rounded ends. These spicules are mostly found in the poral area, where they form a polygonal network (Plate 11, figs. 17, 18), but they also occur in the layer beneath the dermal scales.

(*b*) Nearly sagittal, and irregular triradiates with unequal rays and angles (Plate 11, figs. 19, 23, 24). Plate 11, fig. 19, shows a spicule with rays 130, 98, and 65 μ in length, by 10 μ thick. Plate 11, fig. 23, shows a form transitional between an ordinary triradiate and a tuning-fork.

(*c*) Tuning-fork spicules, found isolated or united into little "fibres" of two or three below the dermal scales. These spicules vary greatly in form, some being very *bizarre* (Plate 11, figs. 20–22, 25, 26). Fig. 20 has the short prongs curved and bent inwards, the total length is 87 μ, the prongs being 18 μ long and 10 μ thick at the base; in another example one prong is only 16 μ long, and the other 65 μ. Spicules *a*, *b*, *c*, form a thin scattered layer between the dermal scales and main skeletal framework.

Plate 10, fig. 6, shows the under surface of a scale with a few "fibres" or bundles each composed of two or three tuning-forks adhering to it.

The remarkable *dermal scales* or plates are imbricated so that the free edges are directed either towards the oscule or the poral groove (Plate 10, fig. 3B). Some, however, are wholly uncovered by others. The scales are mostly nearly circular in outline, though some are oval and a few triangular with rounded angles (Plate 10, fig. 6, and Plate 11, figs. 10, 11).

They vary considerably in size, a large nearly circular one being 525 μ in diameter and 20 μ thick in the centre, and with sharp, thin edge. A smaller oval one measured 375 × 300 μ, with a thickness of 15 μ.

The scales have commonly a convex outer surface marked with smooth tubercles and ridges, and a flat smooth inner surface, so that they are plano-convex. The inner surface is so remarkably transparent as to be almost invisible, the scale appearing like a thin concave object, but foreign particles on the inner surface or, still better, a vertical section show that it is a thick plano-convex disc.

On the inner surface of some of the scales an epithelial layer of cells with round nuclei was clearly seen; and a few such cells were found on the outer surface, which latter was often covered with the hyphæ or threads of some fungus or alga. In the vertical section of a stained, decalcified portion of sponge the outline of folds which included the scales could be distinguished. The scales are very probably formed by dermal folds of epithelium, and not as products of single cells.

Systematic Position.

Murrayona differs greatly from any previously described Pharetronid sponge. In spite of the absence of spicules from its main skeleton, it clearly shows affinities with the Lithoninæ, especially with *Petrostroma schulzei*, Död., in having bundles of tuning-forks in its scattered and scanty sub-dermal layer of spicules, and in having a firm main skeletal framework. The Lithoninæ have an axial core of spicules surrounded by cement. In *Murrayona*, apparently, the sponge has given up forming this axial core. I had stated (5, p. 510) concerning *Merlia normani*, K., which I had at first supposed to be a Pharetronid sponge:—"In the sub-family Lithoninæ the framework is constructed on the *béton armé* principle; in Merlinæ the *béton* is not *armé*, the axial stiffening of spicules being dispensed with."*

What was said of Merlinæ applies to the new sub-family Murrayoninæ, within which it is proposed to place *Murrayona*. Among the siliceous Chalinid sponges the skeleton is formed of horny fibres with an axial core of siliceous spicules. In many instances gradations can be traced from such sponges to purely horny sponges from which the core of spicules has disappeared. The same principle holds as in *Murrayona*. The spongin-cement was, in such cases, originally formed to hold together the bundles of spicules, the latter being finally dispensed with.

The reasons in favour of the theory put forward above concerning the body-skeleton of *Murrayona* seem to me very strong. In the Lithonine sponges *Petrostroma schulzei*, Död., and *Minchinella lamellosa*, Kirkp., the firm skeletal network is constructed of large four-rayed spicules cemented together. In the older parts of the skeleton the calcareous cement becomes more and more developed, so that it is difficult to trace the spicules in the thick strands of the network.

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* I now have the clearest evidence that *Merlia* is a sponge with a siliceous and calcareous skeleton, and that it is in no way related to the Pharetron sponges.

In fact the *rôle* of the cement becomes increasingly important, and that of the axial core of spicules practically negligible. As the cement became more rapidly and abundantly deposited, it would become increasingly difficult for the enclosed scleroblasts to retain their functions.

The dermal scales may have arisen owing to the tendency of so many Pharetronid sponges to form wrinkles and folds, a tendency so marked that Zittel (10, p. 63) gives it in his diagnosis as one of the characters of the group—"a smooth or corrugated dermal layer frequently present." Sometimes the wrinkles are concentric. The tendency to form these calcareous surface-plates may be correlated with the richness of the sponge in cement-building cells.

Murrayona is of interest to the palæontologist, because he must take into consideration the possibility that some fossil Pharetrones, in which he has been unable to detect spicules, may never have possessed them. Hitherto, the absence of spicules has been attributed to fossilisation, and this may be the true cause in many instances, especially in those cases where the spicules are found in some parts of the sponge and not in others.

In *Corynella* (*Myrmecium*) *gracilis*, Münster, the skeletal network consists of thin anastomosing fibres, in which neither Zittel nor Hinde have been able certainly to recognise spicules, though certain wavy lines and other doubtful markings were regarded possibly as being spicules. Zittel (9, Plate 12, fig. 5) gives a figure of a section of *C. gracilis*, showing the fibres with spheroidal rayed crystalline structure. The fibres of *Murrayona* have fan-shaped or circular groupings of fibrillar markings, rather than spheroidal (Plate 11, fig. 16).

The diagnoses of the new sub-family, genus and species, are as follows:—

Sub-family Murrayoninæ (n. sub-fam.), Pharetronidæ with a firm main skeletal network entirely devoid of spicules.

Murrayona (n. gen.), Murrayoninæ with a dermal covering of scales.

Murrayona phanolepis, n. sp. With solely triradiate spicules, and with the poral area limited to a semicircular equatorial groove.

The six known recent species of Pharetronidæ are the following:—

Family Pharetronidæ, Zittel.

Sub-family i. Dialytinæ, Rauff.

With spicules not cemented, but united into strands or bundles, which may be separate or may anastomose to form a network.

1. *Lelapia australis,* Gray. Australia (shallow).
2. *Kebira uteoides,* Row. Red Sea (shallow).

Sub-family ii. Lithoninæ, Zittel.

With spicules cemented together to form a solid network.

3. *Petrostroma schulzei,* Döderlein. Japan, 109–218 fathoms.
4. *Plectroninia hindei,* Kirkp. Funafuti, 50 fathoms.
5. *Minchinella* (? *Rhaphidonema*) *lamellosa,* Kirkp. New Hebrides, 70 fathoms.

Sub-family iii. Murrayoninæ, n. sub-fam.

With firm main skeletal network devoid of spicules.

6. *Murrayona phanolepis,* n. gen., n. sp. Christmas Island, 46 fathoms.

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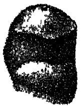

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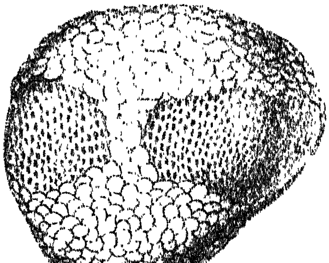

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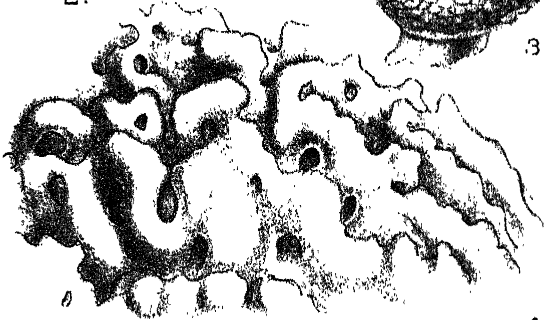

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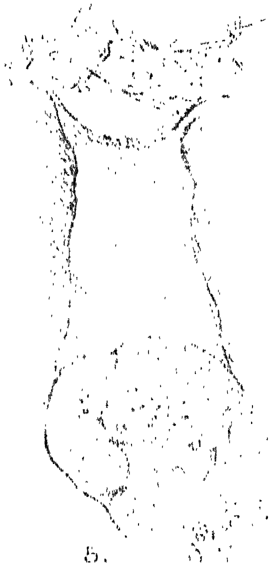

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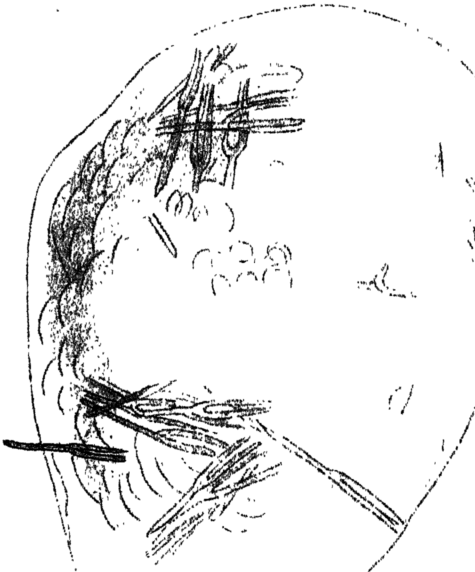

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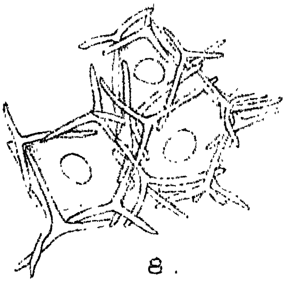

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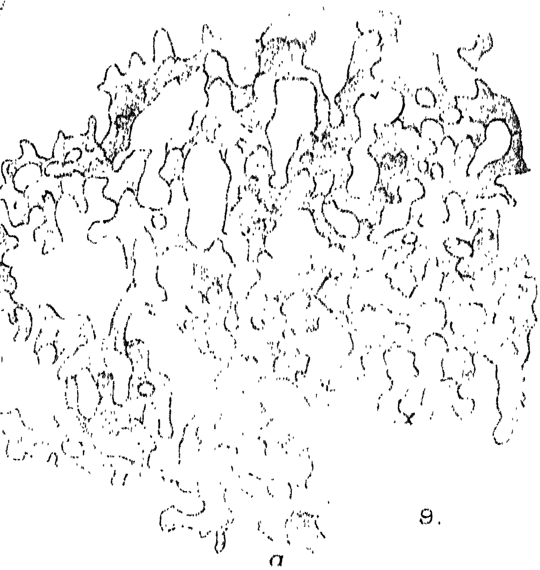

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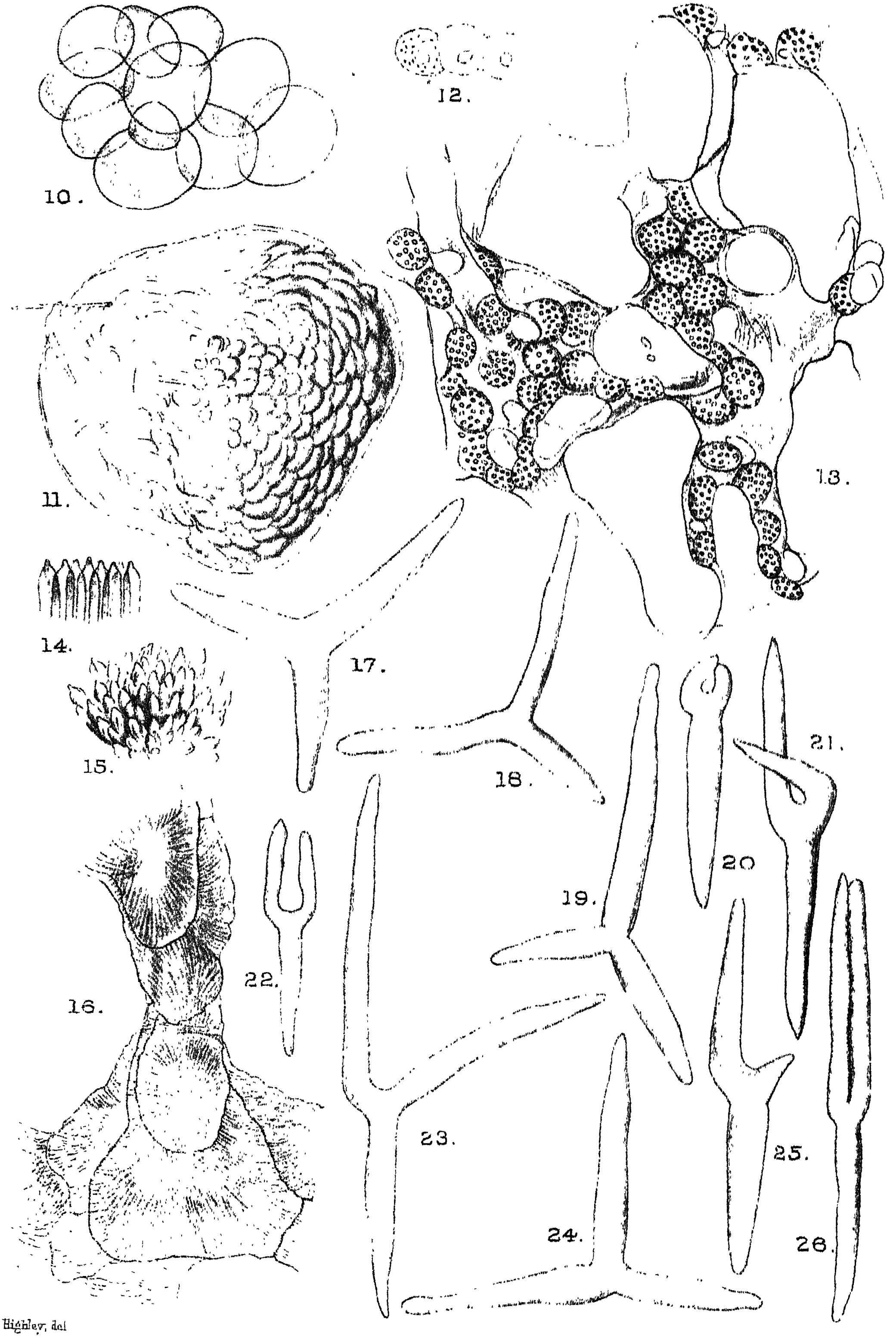

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EXPLANATION OF PLATES.

PLATE 10.

Fig. 1, 2.—*Murrayona phanolepis*, gen. et sp. nov. Specimens B, C. Nat. size.
„ 3.—Specimen A, showing oscule. Nat. size.
„ 3A.—The same, showing pore-area, inside view. Nat. size.
„ 3B.—The same, showing pore-area and scales. × 5.
„ 4.—Surface of portion of firm skeletal framework. × 20.
„ 5.—Fragment broken off from pore-area, showing surface-pores, a sub-dermal space, and a surface-cluster of flagellated chambers, with prosopyles and—on deeper plane—apopyles.
„ 6.—Under surface of a dermal scale, showing tuning-forks loosely adherent. × 125.
„ 7.—Broken fragment of same. *a*, canal. × 20.
„ 8.—Portion of pore-area, showing 3 pores. × 125.
„ 9.—Ground-down section of skeletal network. *a*, oscular region. × 12.

PLATE 11.

Fig. 10.—Dermal scales. × 12.
„ 11.—Dermal scale. × 125.
„ 12.—Flagellated chambers, showing apopyles. × 125.
„ 13.—Section of canals and flagellated chambers. × 125.
„ 14.—Section of a strand of the firm framework, showing fibrillæ. × 1150.
„ 15.—Surface of a strand of same, showing ends of fibrillæ. × 1150.
„ 16.—Portion of skeletal framework, showing the flake-like structure and fibrillæ.
„ 17, 18, 19, 23, 24.—Triradiate spicules. × 425.
„ 20, 21, 22, 25, 26.—Tuning-fork spicules × 425.

The Proteolytic Enzyme of Drosera.

By Jean White, D.Sc., Victorian Government Research Scholar at Melbourne University.

(Communicated by D. H. Scott, F.R.S., December, 1909, and subsequently withdrawn. Recommunicated September 6,—Read November 17, 1910.)

It has been known for many years that if pieces of raw meat be placed on the leaves of a living Drosera plant, the meat becomes decomposed, and the nutritious parts disappear, having been apparently absorbed by the plant. The decomposition was assumed to be brought about by means of proteolytic enzymes which were supposed to be present in the excretion of the glands with which the foliage leaves are abundantly supplied.*

As a general rule, some or all of the leaves of a plant growing under normal conditions are found to contain insects in various stages of decomposition, which have been caught and entrapped by the sensitive glandular hairs curling over their bodies and thus imprisoning them. It is found that the bodies of these insects undergo the same changes as the bits of raw meat did. The hairs do not unclasp the insect's body until all the nutritious matter which it contains has disappeared, and they only release their hold when the remaining parts are simply skeleton, wings, etc.

This decomposition of the body of the insect does not necessarily imply that proteolytic enzymes are excreted by the gland cells of the plant, for it is quite possible that the solution of the insect's body, and of the pieces of raw meat, may be accomplished by the action of putrefactive, or at least of enzyme-producing, bacteria. Tischutkin states that it is solely bacterial digestion which occurs in carnivorous plants.†

The same idea was put forward by Dubois‡ in his paper "Sur le prétendu pouvoir digestif du liquide de l'urne des Nepenthes."

The only record I could find of experiments performed for the purpose of precipitating the proteolytic enzymes, if actually present, in the glandular excretions of Drosera was that of Lawson Tait.§ In 1875 he isolated a proteolytic ferment from the glandular secretion of *Drosera dichotoma*, which he states closely resembles pepsin. The method he adopted for the preparation of the ferment was as follows:—

"The secretion from the Drosera was collected on a feather, which was then washed in pure distilled water. The solution was acidulated with dilute phosphoric acid, and then a thin mixture of chalk and water was added, drop by drop, until the effervescence ceased. The mixture was allowed to stand for 24 hours, when the clear fluid was removed.

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* 'The Soluble Ferments and Fermentation,' Reynolds Green, p. 214.

† 'Bot. Centralbl.,' 1892, vol. 50, p. 304, and 1893, vol. 53, p. 322.

‡ 'Compt. Rend.,' 1890, vol. 111, p. 315.

§ 'Nature,' 1875, vol. 12, p. 251.

"The precipitate was treated with very dilute hydrochloric acid, and the result treated with a saturated solution of pure cholesterin prepared by Bencke's method in a mixture of absolute alcohol and absolute ether. The mass which separated was then dissolved in absolute ether, and in the resulting water was suspended a greyish flocculent matter which was found to be perfectly amorphous. It was dried at a temperature of 42° C. When applied to a small quantity of fresh milk, the latter was observed to become coagulated."

This method appears to be exceedingly cumbersome, and the results to be unsatisfactory from the standpoint of proteolytic enzymes, as the ferment isolated was only noted to cause a viscid coagulation in milk. Besides, it is always possible that the cholesterin used might be contaminated with traces of various kinds of enzymes.

Vines* precipitated a proteolytic enzyme from the pitchers of Nepenthes in the following manner:—To a certain volume of liquid obtained from the pitchers an equal volume of absolute alcohol was added. To increase the bulk of this precipitate phosphoric acid and then lime water were added. Ammonium carbonate was added until the liquid became neutral, and the precipitate was filtered off. Some of the precipitate was shaken up with 0·2-per-cent. solution of HCl, and this liquid was again filtered, when the clear filtrate was found to actively digest fibrin.

In order to determine the true state of affairs concerning the proteolytic enzymes of Drosera, if any such do exist, I performed a series of experiments in which the enzymes were precipitated from the fresh leaves of the following species of Drosera:—*D. auriculata, D. Menziesii, D. peltata, D. Whittakeri.*

The method adopted for their precipitation was practically the same as was employed in connection with an investigation of the enzymes and latent life of seeds.† The leaves were cut off from the plants, and all traces of foreign matter were removed from them. They were then washed in cold boiled water, and again in a strong solution of chloroform, which acts as an antiseptic; in this solution they became quite flaccid. The leaves were now chopped up into minute pieces with a sterilised knife, and the fragments weighed and put into a bottle containing about 100 c.c. of lukewarm boiled water and about 30 drops of chloroform. The bottle was shaken vigorously for two hours, when its contents were filtered.

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* 'Ann. Bot.,' 1897, vol. 11, p. 573.

† 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 424.

To the filtrate was added half its volume of saturated solution of ammonium sulphate, which was found to be far more efficient in this instance for precipitating the proteins and enzymes than methylated spirit. The precipitate produced in this way was filtered off on to filter paper which had been previously sterilised with boiling water, and was dried in a sulphuric acid desiccator. When almost dry, the precipitate was scraped off the filter paper with a sterilised knife and dissolved in cold boiled water.

A small quantity of this solution was put into four test-tubes, A, B, C, and D. The contents of A were boiled, and into A and B was put a little well washed and teased fibrin. The contents of C were also boiled, and into C and D was put a small amount of Witte peptone. About 20 drops of chloroform were poured into each test-tube, and each tube was carefully shaken up. The ends were plugged with cotton wool, and the tubes placed in an oven at 35° C. Samples of the freshly made solution were tested for the biuret and tryptophane reactions, and it was found that distinct traces of peptone were discernible, but there was no sign of amido bodies in the solution.

After a period of time varying from about 30 to 50 hours, the test-tubes A, B, C, and D were removed from the oven, and their contents were tested for evidences of the activity of proteolytic enzymes. Application of the biuret test to tubes A and B disclosed the presence of a considerable quantity of peptone in B, while traces were detected in A in about the same proportion as existed in the freshly made solution.

To tubes C and D, the tryptophane test was applied, with the result that there were not the minutest signs of the presence of any amido bodies.

Results of Experiments.

Species.	Time of digestion.	Reaction to biuret.	Reaction to tryptophane.
D. auriculata............	50 hours	Good reaction	No reaction
D. Menziesii	40 "	"	"
D. peltata	40 "	"	"
D. Whittakeri	40 "	Very good reaction	"

The results of this series of experiments, most of which were performed several times with different specimens of the same species of Drosera, serve to indicate that apparently peptone is the ultimate product of the digestion of proteins by the digestive fluids of Drosera. It also shows that peptones are present in small amounts in the extract prepared from the leaves of the different species of Drosera, even in the ammonium sulphate precipitate, and that the amount is very rapidly increased if the extract is left at a fairly high temperature in contact with fibrin.

This latter fact points to the existence of a pepsin-like enzyme which is apparently present in considerable quantities, and is not in the form of a zymogen. This latter statement agrees with that of Vines, who in his previously mentioned paper on the proteolytic enzymes of Nepenthes, came to the conclusion that the pitcher liquid of Nepenthes contains no zymogen.

As the digestive process does not appear to extend beyond the production of peptones, it seems as if no traces of other proteolytic enzymes such as erepsin or trypsin are present; this does not altogether coincide with the results obtained by Vines in his researches on the pitcher liquid of Nepenthes.

In accordance with his results, I have made very many attempts to find even the minutest signs of protein digestion carried to the amide stage, but have always been unsuccessful. I have been therefore forced to the conclusion of the non-production of these bodies in the species of Drosera mentioned, when growing under their normal conditions in the Victorian bush. It is in fact hardly to be expected that all carnivorous plants should carry out their digestion in exactly the same way and by the same means.

In order to ascertain whether the leaves were capable of absorbing peptones, I performed the following simple experiment.

A saturated peptone solution was prepared with carefully sterilised water, and after filtering it, two drops of the clear solution were applied to five similar marked leaves (which were apparently perfectly horizontal) of plants of *Drosera Whittakeri* which were growing in a flower-pot. One of these leaves was cut off at once, and also a similar leaf to which no peptone solution had been added. The pot was now covered over with a large bell jar, the air inside which was kept damp.

After leaving the flower-pot undisturbed for five hours the marked leaves were cut off and put into sterilised water and the biuret test applied. But in no case was any peptone reaction discernible, thus showing that, while all the water from the solution placed on the leaves had not completely disappeared, the peptone dissolved in the solution had done so, having been absorbed by the leaves of the plant.

Careful microscopic examination was made of the leaves after being cleaned, and also of those in which the remains of the insects' bodies were still present, but no signs of bacteria were visible, so that it seems most probable that the digestive fluid exerts an antiseptic action.

Since the above was written, Prof. Vines was so good as to give me the benefit of certain suggestions and criticisms during a visit made to England, and these were such as to render it advisable to repeat certain of the experiments under different conditions and with varying controls. For this purpose *Drosera Whittakeri* was selected.

Prof. Vines suggested that the strong chloroform used as an antiseptic might exercise an inhibitory effect on the enzyme action. To ascertain if this were the case, the experiments were repeated just as before, with the exception that 4 drops of 0·1-per-cent. HCN were added to each 100 c.c. of the digestive solution in the test-tubes, in place of the chloroform previously employed as an antiseptic. After leaving the test-tubes for 40 hours in an oven at 32° C. I found that the same results were arrived at as before, *i.e.* that marked fibrin digestion had occurred, but no trace of any digestion of peptones could be detected.

Prof. Vines further mentioned that the reaction of the digestive solution might affect the activity of the enzymes present in it. In order to test this point, the second additional series of experiments was performed. The freshly made digestive solution was found to be neutral, and into each of 12 test-tubes (A—L) was poured 100 c.c. of this neutral solution, and four drops of 0·1 per cent. hydrocyanic acid. The contents of each test-tube were then treated as follows:—

The contents of test-tubes A, B, C, D, were left neutral. The solution in B and D was boiled. To A and B was added a little well washed fibrin. To C and D a small quantity of Witte peptone was added. The contents of E, F, G, and H were acidified by the addition of a few drops of 0·2-per-cent. hydrochloric acid. The solution in F and H was boiled. To E and F fibrin, to G and H Witte peptone was added. The contents of I, J, K, L were rendered alkaline by pouring into each test-tube a few drops of a 1-per-cent. solution of sodium carbonate. The solution in J and L was boiled. To I and J fibrin, to K and L Witte peptone was added.

The test-tubes were then plugged with sterilized cotton wool and put into the oven at 32° C. as before. After 40 hours the contents of all the test-tubes were tested and it was found that A, E, and I all gave a strong peptone reaction, the strength of which was apparently the same in each; B, F, and J gave a faint peptone reaction of the same strength as the original freshly made solution exhibited, showing that in these boiled samples, no further protein digestion had taken place.

On application of the tryptophane test to the contents of the test-tubes C, D, G, H, K, and I, it was found that in none of the tubes could there be detected the faintest trace of amido bodies or of any digestion carried beyond the peptone stage, and the same result was obtained when the tubes had been put back into the oven for an additional 24 hours.

The results of these additional experiments must consequently serve to strengthen my previous conclusion that the digestive process by the proteolytic enzyme present in the leaf glands of Drosera does not extend further than the production of peptones.

In conclusion I wish to thank Prof. Ewart for all the help he has given me. Supplies of fresh material of the different species tested were obtained as they were required by Mr. J. W. Audas, of the National Herbarium.

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Further Results of the Experimental Treatment of Trypanosomiasis; being a Progress Report to a Committee of the Royal Society.

By H. G. Plimmer, F.R.S., W. B. Fry, Captain R.A.M.C., and H. S. Ranken, Lieutenant R.A.M.C.

(Received September 19,—Read November 3, 1910.)

The following results are a continuation of the work of which summaries have already appeared in the 'Proceedings of the Royal Society.'*

These experiments have been carried out with the same strain of Surra as was used before, at the Brown Institution, and at the Elstree Farm of the Lister Institute.

Further Experiments with Antimony upon Dogs by New Methods.

In the last Report† we summarised the results obtained by treating dogs suffering from Surra with antimony. We found that in some dogs the subcutaneous or intramuscular administration of antimony or of its salts caused inflammatory swellings and often abscesses, with much constitutional disturbance, and therefore, although certain good results had been obtained, the method seemed impracticable for the treatment of animals upon a large scale. We then tried the effect of *intravenous* injections of the salts of antimony and even of the metal itself, and this would seem at present to be the most promising method of giving antimony, for, if the injection of the metal into the veins be carried out successfully no irritation is caused. If the salts be injected in solution the elimination is so rapid that no good result can be obtained in the acute form of trypanosomiasis with which we have been working, so the actual injection of the metal itself has been successfully carried out in now a large number of instances. In the last two Reports mentioned above experiments have been recorded with antimony (metal) in a state of extremely fine division, and it was shown that it was relatively much more powerful than the salts in its action upon trypanosomes, and that its effects lasted much longer than those of the salts. The particles of metal, which are very minute—they vary roughly from 0·5 μ to 2·5 μ—are taken up by the leucocytes, and some compound which is soluble in the *liquor sanguinis* is apparently formed by them. We are not certain what happens: but in rabbits stained films of blood, taken at various intervals after the intravenous administration of the metal, seem to show that the leucocytes go on taking up the particles until they can contain no more, and then disintegrate at varying periods afterwards, thus providing a constant, though small, supply of antimony. The disintegration would seem to be due to the destruction of the leucocytes by the antimony they have taken up, but on the other hand, many of the leucocytes which have not taken up too much appear to be quite healthy. We have found that both cerebro-spinal fluid and blood serum are, as far as a solution of any trypanocidal substances is concerned, by themselves quite without action on metallic antimony, even when kept in contact with it for periods up to three days. Experiments were undertaken with these fluids after their contact with the metal both on infected animals and upon trypanosomes *in vitro* with entirely negative results.

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* 1907, B, vol. 79, pp. 500—516; 1908, B, vol. 80, pp. 1—12, and 477—487; 1909, B, vol. 81, pp. 354—371.

† 'Roy. Soc. Proc.,' B, vol. 81, p. 367.

The time at which no more particles can be found in, or in which they disappear from, the leucocytes, varies in the different animals: in rabbits they can be found for three or four days; in horses we have not found them after three days; and in dogs not after two.

Sufficient soluble antimony is formed in all these animals after an appropriate dose of the metal to drive the trypanosomes out of the peripheral blood in about 2½ hours; that is, more than double the time taken by the soluble salts to attain the same result.

We have not had in rabbits, dogs, or horses any plugging of capillaries with the metal, but the animals appear to be more sensitive to over-dosage with the metal than with the salts. Testing the dosage on healthy uninoculated animals is also fallacious, as a fatal dose to a healthy animal is apparently borne well by a similar animal when its blood is full of trypanosomes; we believe, also, that in the same animal a large dose is better borne when the blood contains trypanosomes than when it is free.

When antimony in this form is prepared in larger quantities, it becomes more difficult to remove impurities from it. We mention this as we have had deaths from doses which had previously been well borne, which we attribute to these impurities, but these difficulties are now overcome.

Of the 26 dogs treated with antimony, the details of which are given in the last Report, one (No. 1) lived for over 15 months in good condition, and was then killed. Inoculations from all the organs into rats proved negative, and the animal was regarded as cured; twelve others (Nos. 2, 5, 7, 9, 10, 11, 13, 14, 16, 17, 18, 19), which lived for from 48 to 94 days, if inoculation into rats from the organs after death can be accepted when negative as evidence of cure, may also be regarded as cured.

Average Duration of Untreated Disease, 14 Days.

No.	Weight in kilos.	No. of doses.	Substance used and quantity of dose.	Recurrences.	Remarks.	Gain or loss of weight.	Subinoculations from organs.
						kilos.	
27	9¼	18	6 m. 5 per cent. lith. ant. tart. diluted, intramuscularly	1	*Living* 1 *year* 121 *days after inoculation*...........	+ ½	
28	10¼	9	8 m. 5 per cent. lith. ant. tart. diluted, intramuscularly	1	Died on 75th day from pneumonia	+3	Negative.
29	9¾	10	7 m. 5 per cent. lith. ant. tart. diluted, intramuscularly	1	Died on 84th day from pneumonia	+1	Negative.
30	9	12 3	6 m. 5 per cent. lith. ant. tart. diluted, intramuscularly 50 mg. ant., intravenously	3	Died on 168th day with nervous symptoms, "fits"	−1¼	Positive from C.S.F.
31	8½	9 3	6 m. 5 per cent. lith. ant. tart. diluted, intramuscularly 20 m. 5 per cent. lith. ant. tart. diluted, intravenously	2	Died on 87th day: kidneys inflamed	−1	Negative.
32	11	4 2	20 m. 5 per cent. lith. ant. tart. diluted, intravenously 80 mg. ant., intravenously	1	Died on 154th day, 3 months after last dose. Spleen large	+1¼	Negative.
33	9	2 4	20 m. 5 per cent. lith. ant. tart., intravenously 60 mg. ant., intravenously	1	Died on 116th day. Trypanosomes in blood	− ½	Positive.
34	8¼	2 4	20 m. 5 per cent. lith. ant. tart., intravenously 80 mg. ant., intravenously	3	Died on 81st day from pneumonia.....................	−1¼	Negative.
35	9½	2	80 " "	—	*Living* 347 *days after inoculation*....................	+1	
36	13	4	90 " "	1	Died on 88th day. Trypanosomes in blood.........	− ½	Positive.
37	9	4	80 " "	—	Died on 67th day with nervous symptoms	−1¼	Negative.
38	7	3	60 " "	2	Died on 40th day. Probably poisoned with ant.	−1½	Negative.
39	9½	5	60 " "	2	Died on 76th day. Probably poisoned with ant.	+1	Positive.

The preceding Table shows in outline the results with 13 other dogs since treated with antimony in the method indicated.

The last two dogs probably died from the effects of antimony, as other dogs at the same time died after the first dose of that particular sample.

Dogs are particularly susceptible to both Surra and antimony, but if we accept negative sub-inoculations made from the organs after death as fair evidence of cure, this table and the previous one will work out as follows:—

In the first Dog Table (in the last Report), on this assumption, out of 26 dogs, 13 may be regarded as cured, and in the one above, 2 are alive and 7 gave negative results, that is 22 out of 39 altogether. The indications are also that treatment with the metal intravenously has yielded better results than that with the salts, either intravenously or intramuscularly.

The technique in dogs is difficult, as only the saphenous veins in the hind legs are available without an operation, and the suspension of the metal in salt solution must be as dilute as possible.

Intraperitoneal Injection of Antimony (Metal).

A series of experiments on the effects of intraperitoneal injections of the metal has been made on rats and rabbits. Both bear the metal quite well in the peritoneum, and neither pain nor inflammation were caused. The metal rapidly disappears from the peritoneum, and after three or four days none can be seen. In rats, one dose of 15 milligrammes will keep the trypanosomes out of the blood for an average of 25 days. No los of weight or appetite occurred. In dogs this method is impracticable, as the injection causes acute peritonitis.

Rats Treated with Arsenophenylglycin and Antimony.

In the last Report is an account of some experiments with arsenophenylglycin. A rat treated with one dose of arsenophenylglycin had a dose of lithium antimonyl tartrate given to it the day *before* recurrence took place in other rats treated with arsenophenylglycin. The rat lived 222 days without recurrence; and, regarded as cured, it was reinoculated and treated intraperitoneally with one dose of antimony (metal): it died 32 days after, from a recurrence. Another rat treated with one dose of arsenophenylglycin and five doses of lithium antimony tartrate died of the disease: in this case the latter drug was not given until *after* recurrence took place.

These experiments show again that no protection is afforded by a previous attack, and also that the time at which treatment is begun is of importance.

New Arsenic and Antimony Compounds.

Five Surra rats were treated with a new arsenic-camphor compound kindly sent to us for trial by Dr. Morgan, of the Imperial College of Science. They died at about the same time as untreated rats, and the substance was found to be too irritating for use.

Eleven Surra rats were treated with a new organic antimony compound, also sent to us by Dr. Morgan. This caused considerable swelling, and did not effect the disappearance of the trypanosomes. The arsenic compound had no effect, the antimony a small effect. *In vitro,* the latter killed the organisms very slowly.

Treatment of Goats with Antimony.

According to Musgrave and Clegg* inoculated Surra in goats is always fatal, and they give 18 days as the time the untreated disease runs.

We have treated two goats with antimony. One has had three doses of about 80 milligrammes each of the metal suspended in egg-yolk and oil subcutaneously, and is living and well 15 to 16 months after inoculation. The other had 15 doses of 20 m. of 5 per cent. lithium antimonyl tartrate, and later, when it became ill with nervous symptoms, four doses of 150 milligrammes of the metal intravenously. It became paralysed and died 236 days after inoculation.

Inoculations into rats from organs and cerebro-spinal fluid were negative.

Treatment of Surra Rabbits with Antimony.

The results of treatment of rabbits with antimony have been striking. They were left until they were very ill with the disease, with sores on the face, swollen genitals, œdema, eyes and nose inflamed, and unable to stand; after treatment all these symptoms disappeared, and their deaths were, we believe, due to poisoning with an impure antimony.

The table on p. 145 sets forth the principal points.

Inoculations made from Nos, 2, 3, and 4 into rats after death were negative.

It will be noticed that after three doses of the salt there was a recurrence, and also after one dose of the metal, but not in those rabbits which had more than one dose of the metal.

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* 'Trypanosoma and Trypanosomiasis,' 1903.

No.	No. of doses.	Material used, and quantity.	Recurrences.	Remarks.
1	3	5 m. 5 per cent. lith. ant. tart., intravenously	1	Died on 116th day. Recurrence occurred on the 115th day, when the impure metal was given.
	1	30 mg. ant., intravenously		
2	3	30 mg. ant., intravenously	—	Died on 80th day.
	1	30 mg. ant., intraperitoneally...		
3	2	60 mg. ant., intravenously	—	Died on 183rd day, probably killed with impure antimony. Had increased 300 gr. in weight.
4	2	30 mg. ant., intravenously	—	Died on 147th day, probably as No. 3. Had increased 180 gr. in weight.
5	1	30 mg. ant., intravenously	1	Died on 79th day. Lost 500 gr. in weight.

Experiments with Surra Rats after One Dose of Antimony.

We have had the following curious experience with infected rats which had been treated with *one* dose of lithium antimonyl tartrate. One dose of this drug causes the disappearance of all trypanosomes from the blood, and keeps the blood free from them for a variable period, the average time of recurrence being 18 days. We wished to find out how soon the blood of a rat thus treated became infective to other rats on sub-inoculation, and whether it would prove to be so before trypanosomes could be found in it by ordinary microscopic examination. Sub-inoculations were therefore made from the treated rat's blood at various intervals from the 2nd to the 16th day after the one dose of antimony. It was then found that the blood of the original rat was infective long before any trypanosomes could be found in the peripheral circulation; but also that this infectivity was not constant, an infection being produced on one day, and none occurring on one or two subsequent days. Moreover, as the table shows, the course of the disease (incubation period especially) was very prolonged. Ordinary untreated infective blood produces in rats a recognisable infection in from two to four days, and death in from six to seven days. In these cases either the trypanosomes could not be found in the peripheral blood at all, the only lesion being the very large spleen, or were not found until the 28th day after inoculation. These prolonged incubation periods appeared to have no relation to the time of the recurrence in the original rat, and the infection was brought about by blood which not only showed no trypanosomes, but appeared to be perfectly normal in structure. We have no explanation of these results, but as all the rats which died had very large spleens we can only suppose that the trypanosomes were present in either some form or place which we did not recognise or find. If this be so the experiments are of interest as showing the influence on the trypanosomes, and the constant effects, of one dose of antimony.

Table giving Details of the above-mentioned Experiments.

Rats inoculated from treated Rat 1 on—	Death occurred on—	Spleen.	Trypanosomes present (+) or absent (0) from blood.	Remarks.
5th day after dose ...	—	—	—	Living 103 days.
7th " " ...	31st day	Very large	0	
10th " " ...	16th day	Large	0	
12th " " ...	—	—	—	Living 95 days.
14th " " ...	13th day	Very large	0	
Ditto from Rat 2 on—				
2nd day after dose...	—	—	—	Living 52 days.
4th " " ...	—	—	—	Living 50 days.
7th " " ...	—	—	—	Living 47 days.
10th " " ...	28th day	Very large	+ +	
16th " " ...	41st day	Enormous	0 in blood, spleen, liver, adrenals.	

It will be noticed that death was delayed up to as long as 41 days. Two other rats were given a relatively smaller dose of antimony, and rats inoculated from these on the 4th, 7th, 9th, and 11th days after the dose died with trypanosomes in their blood on the 24th, 19th, 19th, and 29th days respectively, the incubation period only being much prolonged.

Experiments upon Surra Horses with Antimony.

Inoculated Surra in horses runs a quicker course than that acquired naturally, and it is difficult to give a certain time for the disease, as a good deal depends on the condition of the horse, and experimental horses are usually old and worn-out and would succumb more quickly than young ones. Cases of untreated experimental Surra in horses have died as early as the 14th day, but the time probably depends very much upon the age and condition of the animal. The horses we have used were inoculated from rats, and the incubation period was practically six days. Musgrave and Clegg* give 6 to 13 days as the incubation period, so that our strain of Surra is a fairly virulent one for horses.

Both intramuscular and intravenous injection of antimony (metal) have been tried. Doses of 2 grammes have been given, intravenously, and if sufficiently diluted with warm salt solution no unpleasant symptoms follow. Large doses are best borne when injected into a peripheral vein in one of the legs, and one injection of 1 gramme will keep the blood free from trypanosomes for from 2 to 3 weeks. If the injection be made into the jugular vein considerable depression, and even faintness, may be induced. The use of a peripheral vein involves throwing the horse, which is risky if repeated doses have to be given. We have not yet ascertained the maximum dose. All the horses died with nervous symptoms, and although life has been prolonged (in one case to 121 days) none of them has shown satisfactory results. We have persisted in our experiments with metallic antimony because we hoped that, as it is taken up by the leucocytes, it might possibly be carried by them to, and be deposited in, parts not accessible otherwise; also, because we know that in syphilis the protozoon causing the disease is killed by the injection of metallic mercury We know also that syphilis requires a continued treatment, and it may be that in trypanosomiasis a continued treatment also is necessary. The effects produced by metallic antimony are so striking that we are emboldened to think that if the methods could be improved a much better effect would be obtained.

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* *Op. cit.*

The following Table summarises the experiments upon horses.

No.	No. of doses.	Material and quantity given.	Recurrences.	Remarks.
		Intramuscular—		
1	7	20 per cent. suspension of sod. ant. tart. in Lambkin's medium. Doses from 1 to 20 c.c.; in all 36 c.c.	—	No trypanosomes seen for 17 days before death. Died on 44th day with nervous symptoms and thoracic œdema. Spleen normal.
	1	5 c.c. 5 per cent. ant. in Lambkin's medium		
		Intravenous—		
2	8	Ant. in salt solution. Doses from 0·5 gr. to 1·25 gr., altogether 5·5 gr.	2	No trypanosomes seen for 19 days before death. Died on 52nd day with nervous symptoms.
3	8	Ant. in salt solution. Doses from 0·1 gr. to 2 gr., altogether 9·75 gr.	3	No trypanosomes seen for 20 days before death. Died on 121st day with nervous symptoms.
4	6	Ant. in salt solution. Doses from 0·1 gr. to 2 gr., altogether 4·5 gr.	—	No trypanosomes seen after first dose. Died on 56th day with nervous symptoms. Had persistent thoracic œdema.

With the exception of No. 1, these horses were old and worn-out.

It will be noticed that none of these horses had trypanosomes in the blood for some time before death; No. 3 appeared to be quite well up to the 90th day, and No. 4 up to the 40th day, and both could apparently have worked up to these times. In all cases death was preceded by a very sudden aggravation of symptoms referable to the nervous system.

We considered that treatment by injection into the cerebro-spinal space was impracticable in horses owing to the distance of the spinal canal from the surface, and to the fact that the spinal cord extends much further down the canal than in man.

Experiments with Silver Salts.

Three Surra rats were treated with protargol in doses varying from 0·5 c.c. of a 1-per-cent. solution to 1 c.c. of a 2-per-cent. solution.

Three Surra rats were treated with argyrol in similar doses.

Three Surra rats were treated with silver nitrate in doses varying from 1 c.c. of a 0·02-per-cent. solution to 1 c.c. of a 1-per-cent. solution.

All these rats died at the usual time: death was not delayed, nor were the trypanosomes reduced in number in the blood. *Post-mortem* examination showed that they had died with the usual signs of acute trypanosomiasis, with very large spleens, etc. At the sites of injection of protargol there was œdema and brown staining of the tissues in a circumscribed area: there was more marked œdema and swelling after injection of argyrol, and œdema and localised necrosis of the tissues after injection of silver nitrate. It would appear that the silver salts are not absorbed from the site of injection. Presumably they have a primary cauterising action on the tissues, and are quickly transformed into silver chloride, which is inert.

In vitro experiments were carried out with these three compounds, all of which are soluble in distilled water. Equal sized drops of blood containing trypanosomes and of the solutions were mixed, and the results observed under the microscope.

Protargol solutions from 1/200 to 1/10000 produced very little effect; in 1/200 dead trypanosomes were seen in 30 minutes, but many were alive in 24 hours. Silver nitrate in solutions from 1/200 to 1/10000 has much the same effect as protargol.

Some trypanosomes are killed in 30 minutes by the stronger solutions, but the remainder are as active as the controls up to 24 hours and longer.

Argyrol (which contains 30 per cent. silver) in solutions of 1/1000 to 1/10000. The stronger solutions are still less active than those of protargol and silver nitrate; and the more dilute have no effect up to three hours. Thus it appears that these bodies have no effect except the early effects of the stronger solutions; they probably combine with the salts in the blood-plasma and corpuscles, and become inert.

Citrate of silver in its strongest solution of 1/4000 is also inert.

The Oxidation of Phenol by Certain Bacteria in Pure Culture.

By Gilbert J. Fowler, Edward Ardern, and William T. Lockett.

(Communicated by Dr. A. Harden, F.R.S. Received September 30,—
Read November 17, 1910.)

In the course of investigations on the effect of various antiseptic substances on bacterial sewage filters, it was found that solutions of pure phenol could be oxidised on filters of this description. It was further found that the phenol apparently exerted a selective action on the bacteria present in the filter, only three or four types of organism appearing in the filtrates.

Of these about half were liquefying organisms, identified as *B. liquefaciens fluorescens*. At first it was thought that the main non-liquefying, or extremely slowly liquefying, organism was *B. fluorescens non-liquefaciens*, on account of the apparently fluorescent yellow colonies it produced; subsequent investigation showed that this was not the case (see note at end of paper). It is therefore referred to in the paper simply as the chromogenic organism.

On passing a current of filtered air through the liquid containing a mixture of these organisms, together with small quantities of phenol, the latter was slowly oxidised, which was not the case in a control experiment where organisms were absent.

Preparation of Pure Cultures.

The survival of the particular organisms—*B. liquefaciens fluorescens* and the chromogenic organism—in the experiments referred to, and their constant presence in filtrates from other filters dealing with pure phenol solutions, led to experiments being made on the separate effect of each of these organisms on phenol in solution.

Colonies of the organisms in question were separated; the liquefying variety was usually grown on gelatine, the chromogenic organism in broth.

Cultures were obtained from different sources—from the plates made in connection with the previous experiment, and from plates made from filtrates from different filters. Later, further cultures were obtained from plates showing colonies of only one organism.

The culture medium used had the following composition:—

Broth.—	Per litre of water.
Peptone	10 grammes.
Meat extract	5 or 10 „
Sodium chloride	5 „

Nutrient Gelatine.—Medium made by addition of 10 per cent. of gelatine to broth obtained as above.

Diluted Broth Solution.—Made either by the addition of about 10 c.c. of broth culture to 500 c.c. of sterile water and further incubation, or by inoculating a quantity of sterile water, containing a few cubic centimetres of "broth," with the organism.

Special Medium.—This medium is similar to one used by Percy Frankland in his earlier experiments, and had the following composition:—

	In 1000 c.c. of water, with 4 grammes of pure calcium carbonate in suspension.
Potassium phosphate	0·1 gramme.
Magnesium sulphate (cryst.)	0·02 „
Calcium chloride (fused)	0·01 „
Glucose	0·3 „
Peptone	0·25 „

Action of Pure Cultures on Phenol.

The preliminary experiments made in connection with the action of each of the organisms on phenolic solutions were carried out in 40-oz. bottles, fitted with rubber stoppers, tubes, etc., and arranged in series in a similar manner to the preceding experiment.

The procedure generally adopted was as follows:—To a sterile phenol solution (vol. 500 c.c.) containing 0·01 to 0·02 per cent. of phenol, a quantity of the culture of the particular organism under examination was added, the solution aërated and tested from time to time.

The experiments throughout were conducted in such a manner as to prevent as far as possible any air or other infection. It was not supposed that the solutions were kept entirely free from infection, the extent of the contamination presumably increasing with the length of duration of experiment.

I. *Action of* Bacillus liquefaciens fluorescens *on Phenol.*

Several experiments were made in connection with this organism. Generally a few cubic centimetres of liquefied gelatine containing the organisms were added to a pure phenol solution, and in one case to a solution of phenol with added nitrate and nitrite. Practically no change, however, was noted in the phenol content of these solutions after two months' aëration.

The numbers of organisms present at the commencement of experiments were several millions per cubic centimetre, and after two months a considerable number in active condition were still to be found in the solutions.

II. *Action of the Chromogenic Organism on Phenol.*

(a) *Broth Cultures.*—Experiments were first conducted with broth cultures of this organism. A few cubic centimetres of a broth culture were added to a pure phenol solution.

For two or three days no change was noted in the solutions; subsequently, however, the disappearance of phenol was rapid, the whole being completely oxidised in five or six days from the commencement of the experiments.

Typical example:—

		Oxygen absorbed from acid permanganate solution in 3 minutes. Parts per 100,000.
April 9.	Experiment commenced	25·20*
„ 12		25·80
„ 14		2·00
„ 15		1·57

* Equivalent to 16·5 parts of phenol per 100,000.

The 3 minutes oxygen absorption test was chosen as the most convenient for the detection of phenol and its approximate estimation. The accurate determination of phenol in such dilute solutions would be a long and tedious process, while the amount of oxygen absorbed by the constituents of the broth or its products of decomposition bears a very small proportion to the amount taken up by the phenol.

(b) *Diluted Broth.*—An experiment was made using about 30 c.c. of a diluted broth culture in place of ordinary broth. Again it was noticed that no change took place for a few days, followed by rapid disappearance of phenol. The time taken to complete the experiment was eight days, or rather longer than with ordinary strength of broth.

Two experiments were afterwards made with diluted broth cultures (35 c.c.) with the addition of a few cubic centimetres of ordinary sterile broth. As before, no change was noted in the earlier stage of the experiment, but after four days only, complete oxidation was rapidly brought about in 24 hours. The air passing through these solutions was previously sterilised by bubbling through mercuric chloride solution.

Example:—

		Oxygen absorbed from acid permanganate in 3 minutes. Parts per 100,000.
June 27.	Experiment commenced	24·8*
„ 29		24·8
„ 30		24·8
July 1		22·8
„ 2		1·2

* Equivalent to 16·2 parts of phenol per 100,000.

It appears, therefore, that the action is accelerated by the addition of further nutrient material.

(c) *Cultures on Special Media.*—A few cubic centimetres of one of these cultures were added to a phenol solution. The disappearance of phenol in this case was gradual, commencing in the first day or two—the time taken, however, for oxidation was considerably longer than in the case of the broth cultures, viz. 16 days.

Results:—

	Oxygen absorbed from acid permanganate in 3 minutes. Parts per 100,000.
June 29. Experiment commenced	20·8*
July 1	20·4
„ 2	18·0
„ 9	7·9
„ 14	1·4

* Equivalent to 13·6 parts of phenol per 100,000.

Gelatine plates were made from the solutions and it was found, generally, that the numbers of the chromogenic organism present in the solutions, being several millions per cubic centimetre at commencement, were considerably increased during the experiment.

Proportionally, the number of organisms other than the chromogenic present in the solutions were few.

(d) *Addition of a Culture of the Chromogenic Organism to a Solution previously unacted upon by* B. liquefaciens fluorescens.—An interesting bottle experiment was made as follows:—To a solution of phenol previously unacted upon by a culture of *B. liquefaciens* as described in the preceding, a broth culture of the chromogenic organism was added. Within four days of the addition of the second culture almost complete oxidation of the phenol had taken place.

Results:—

	Oxygen absorbed from acid permanganate in 3 minutes. Parts per 100,000.
March 2. Experiment commenced	17·40*
April 12	17·00
„ 12 after addition of second culture	19·20
„ 13	18·60
„ 15	8·11
„ 16	2·46

* Equivalent to 11·4 parts of phenol per 100,000.

Gelatine plates of the solution at end of experiment showed presence of large number of both liquefying and non-liquefying organisms.

Flask Experiments.—Similar experiments to the preceding were made with cultures of the chromogenic organism under rigid sterile conditions. Four flasks (300 c.c. capacity), fitted with rubber stoppers, tubes, etc., and connected in series, were used in each experiment. The inlet of the first flask and the outlet tube of the fourth flask were connected with wide tubes drawn out at one end and plugged with cotton wool.

To each of the four flasks at the outset of experiment was added 150 c.c. of phenol solution of required strength (10 parts per 100,000). The inlet tubes were raised in the stoppers so that a short space was left between the surfaces of liquids and the ends of the tubes. The apparatus connected and complete with the contained solutions was then placed altogether in a steamer and sterilised for three successive days.

A culture of the organism was divided into two equal parts, one half being heated to boiling for a minute or so, and further sterilised by placing in the steamer for three days at the same time as the apparatus described above.

When sterile, the second flask was carefully disconnected from its stopper and sterilised or " dead " culture added, the flask being quickly reconnected. To the third flask the remaining half of the culture which contained the living organisms was added in exactly the same manner. The tubes, which had been raised to prevent " sucking back " of the solutions during cooling periods, were then pushed through the stoppers, care being taken that no portion of the tubes which had been previously exposed to the air occupied a position inside the flasks.

The contents of flasks were then as follows:—

Flask I	Phenol solution alone.
" II	" " + dead culture.
" III	" " + living "
" IV	" " alone.

Air was slowly drawn through the solutions by means of an aspirator (rate 10 to 20 litres per day) in the direction of Flask I to IV. By this means the solution containing the living culture was kept free from air infection. Gelatine plates were made of all the solutions after the experiments, and the following is a general description of the bacteriological results obtained:—

Flask I.—(Phenol alone.) Very little contamination and occasionally none whatsoever. Not more than two organisms per cubic centimetre were present at any time.

Flask II.—(Dead culture.) Free from air infection. Plates of 1 c.c. solution usually made. In one case great numbers of the chromogenic organism were found to be present, due to "sucking back" of some of the liquid contained in the third flask; no other organism found.

Flask III.—(Living culture.) With one exception the liquid contained only organisms of chromogenic type. In the isolated case 20 moulds (per cubic centimetre of liquid) were found to be present, possibly introduced with the culture, or during plating, whilst many millions of the organism under investigation were found to be present.

Flask IV.—(Phenol alone.) Generally contained the chromogenic organism. On one occasion it was entirely free. The presence of organisms in this flask is due to the carriage of organisms by the air from the flask containing the living culture.

Chemical Results.—The following is a typical example of the results obtained:—

	Oxygen absorbed from acid permanganate in 3 minutes. Parts per 100,000.
Before experiment—	
Solution of phenol added to flasks before sterilising	12·80*
After experiment—	
Flask I. Phenol alone	10·80
„ II. „ + dead culture	11·80
„ III. „ + living „	0·50
„ IV. „ alone	11·20

No intermediate tests were made.

* Equivalent to 8·4 parts phenol per 100,000.

While these experiments were not quite so perfectly successful as could be desired, yet sufficient evidence was obtained to show that a pure culture of the organism used was capable of oxidising phenol, whilst no change was noted in the phenol content of solutions containing "dead" cultures. Similarly, no change has been observed in the phenol content of solutions of pure phenol.

The time taken for oxidation varied from two to several weeks. It should be noted that the aëration in these experiments was very much less than that obtained in the "bottle" experiments. The amount of aëration appears to make considerable difference in the rate of oxidation.

Both broth and diluted broth cultures were used in these experiments. The following experiment was, however, conclusive, and confirmed the natural inference drawn from the foregoing. A solution was made containing approximately 0·01 per cent. of phenol, together with the following ingredients:—

	Per litre.
Ammonium sulphate	0·1 gramme.
Potassium phosphate	0·1 „
Magnesium carbonate, in suspension	½—1 „

Four hundred cubic centimetres of this solution were sterilised in a litre flask, plugged with cotton wool, in a steam steriliser for three successive days. When sterile, a few drops of a broth culture of the organism were added, particular care being taken to prevent contamination. It was intended to limit the amount of carbon in the media, it being supposed that the phenol on oxidation would supply the deficiency.

The flask and contents were incubated for several days at 20° C., the liquid being aërated by an occasional shake. The solution was tested after five days with the usual precaution to prevent contamination.

Results:—

	Oxygen absorbed from acid permanganate in 3 minutes. Parts per 100,000.
At commencement	19·20*
After 5 days...........................	8·57
„ 9 „	1·83

* Equivalent to 12·5 parts of phenol per 100,000.

On the ninth day, before submitting the solution to chemical tests, gelatine plates were made. Two plates were made taking 1 c.c. of the liquid, and one plate each for 0·01 c.c., 0·0001 c.c., and 0·000001 c.c.

Bacteriological Results (3 days at 20° C.).

1 c.c.	original, 2 plates	......	Crowded with organisms. All one kind. No liquefying colonies.
0·01 c.c.	„ 1 „	......	Crowded. All one kind. Chromogenic.
0·0001 c.c.	„ 1 „	......	Hundreds of colonies. All one kind. Chromogenic.
0·000001 c.c.	„ 1 „	......	130 colonies. Chromogenic. All one kind.

The solution contained 130,000,000 organisms per cubic centimetre, and there was not the slightest evidence of contamination. It may be safely concluded, therefore, that dilute solutions of phenol are oxidised by the particular organism under investigation.

Experiments are being carried out to determine the exact effect upon the oxidation of phenol by this organism, of the addition of other nutrient substances, Experiment II (*b*) having shown that the addition of broth accelerated the action.

Researches have also been in progress on the bacterial oxidation of sulphocyanates, which appears to be more complex, and certainly less rapidly effected than the oxidation of phenol, inasmuch as when a mixture of the two substances is submitted to bacterial oxidation the phenol is invariably the first to disappear.

[*Note added October 20th,* 1910.—Dr. Sidebotham, of the Public Health Laboratory, Manchester, has kindly identified the chromogenic organism worked with in the above experiments. He describes it as follows:—

Bacillus, non-motile, no cilia; size, $2\,\mu \times 0{\cdot}5$; often in pairs, no chains. Grows best at 22° C.; growth ceases at 30° C.; grain negative.

A. P. B.—Greenish yellow; smooth growth.

G. P. B.—Growth all round stab; growth spreads on surface; central raised pale yellow projection, surrounded by pale pink smooth area; gelatine very slowly liquefied.

Potato.—Very bright lemon-yellow; irregular knobby growth.

Milk.—Colour, brown to pale brown; no acid change.

The characteristics seem to be similar to those of *B. Helvolus* (Zimmermann).

The above characteristics have also been observed by one of us (G. J. F.) in the case of an organism regularly occurring in an experimental filter dealing with liquids containing phenolic compounds.]

A Method for Isolating and Growing the Lepra Bacillus of Man. (Preliminary Note.)

By F. W. Twort.

(Communicated by Leonard Hill, F.R.S. Received September 30,—Read November 17, 1910.)

(From the Laboratories of the Brown Institution, University of London.)

For a number of years different investigators have attempted to cultivate the lepra bacillus of man and the allied organisms found in the rat and other animals. It is not intended in this preliminary note to discuss the numerous papers which have been published from time to time from the various English, Continental, and American laboratories. These papers deal with non acid-fast bacilli, or with acid-fast bacilli growing quickly on ordinary media, which, in the opinion of the writer, are contaminating organisms, and not the true lepra bacillus. So far, no one has produced a culture of acid-fast bacilli isolated from a leper, and showing the characters of the lepra bacillus as found in the tissues of man. It was with the object of obtaining a pure living culture of the lepra bacillus that these investigations were undertaken.

The material used was the nasal discharge and scrapings from a typical leper. The discharge showed large masses of lepra bacilli and a number of contaminating micro-organisms. Firstly, most of the contaminations were killed by placing the discharge in a 2-per-cent. solution of ericolin at 38° C. for one hour as recommended for the isolation of the tubercle bacillus*; then cultures were made from the sediment on to different media and incubated at 38° C.†

All the ordinary laboratory media, including Dorset's egg medium, gave negative results. A number of special media containing extracts of fresh gland and other tissues were tested next, the extracts being freed from any contaminating micro-organisms by passing them through a Doulton white filter; these also gave negative results.

In view of the close relationship between the tubercle bacillus and the lepra bacillus, it appeared highly probable that these two organisms would require the same chemical substances for building up their protoplasm, which could be elaborated from the ordinary media only by the tubercle bacillus. It was thought that if these substances could be supplied, already formed, to the lepra bacillus, it might grow, and the easiest method of supplying these substances would be by adding to some good medium the ground-up bodies of tubercle bacilli containing them. Accordingly, a number of tubercle cultures were taken and inoculated on to Dorset's egg medium; when sufficiently grown the tubes were steamed and the growth of tubercle scraped off the surface, care being taken to avoid the medium containing the waste products of the tubercle growth. The tubercle bacilli so obtained were ground up with glycerine and saline, steamed for half an hour and added to the yolk and white of new laid eggs in the following proportions:—

Eggs, 75 parts; 8 per cent. sodium chloride, 25 parts; mix well and add tubercle bacilli, 1 per cent.; and glycerine, 5 per cent., or less.

The medium was placed in test tubes, heated to 60° C. for one hour, and on the following morning incubated at 38° C. for six hours, and again heated in water bath at 60° C. for one hour, and set in slopes at 85° C.

The ericolinised nasal discharge of a leper was inoculated on to this medium, the inoculated tubes being capped with gutta-percha tissue and incubated at 38° C. After 24 hours the medium absorbed a quantity of the ericolin, so the material was lifted off with a platinum loop and rubbed over fresh tubes. The bacilli grew and were sub-cultured in pure growth, the bacilli growing in sub-cultures as fairly long thin beaded rods; the bacilli were well formed and quite acid-fast. The lepra bacillus inoculated on to this medium at first grows extremely slowly, but later growth becomes faster, marked microscopic evidence being obtained in about four weeks. To the naked eye, growth is only just visible after about six weeks, appearing as a colourless film along the needle track. Attempts to sub-culture on to ordinary laboratory media are always negative.

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* Twort, 'Proc. Roy. Soc.,' B, 1909, vol. 81.

† Subsequent experiments have shown that 37° is a better temperature (November 19, 1910).

Experiments are now being carried out, using other organisms than the tubercle bacillus for making the medium, and also testing various micro-organisms on the medium.

In the near future it is hoped to prepare a vaccine from the ground-up lepra bacilli, for the treatment of man suffering from leprosy. An attempt to grow the rat lepra bacillus on the same medium will also be made if the material can be obtained.

In conclusion, I may note that, working with Mr. Ingram, I have also succeeded in isolating and growing the acid-fast bacillus found in the intestine of cows in Jöhne's disease. The first generation of this bacillus grows often long, with occasional branching and club formation, in sub-cultures it gradually grows smaller, and in the second or third generation is about the size of the tubercle bacillus. The growth is only just visible to the naked eye, and sub-cultures on the ordinary laboratory media show no evidence of multiplication. Jöhne's bacillus grows somewhat more easily than Hansen's lepra bacillus; the bacilli being well formed and quite acid-fast. The cultures were incubated at 40° C. It is hoped, when sufficient material is obtained, to prepare a vaccine for diagnostic purposes.

Further details of these experiments will be published later.

Some Enumerative Studies on Malarial Fever.

By Major Ronald Ross, F.R.S., and David Thomson, M.B., Ch.B., D.P.H.

(Received October 12,—Read December 8, 1910.)

Prefatory Note by R. Ross.—Towards the end of last year the Advisory Committee for the Tropical Diseases Research Fund (Colonial Office) allotted considerable funds to the Liverpool School of Tropical Medicine for researches to be carried out in Liverpool. This enabled us to commence, under my direction, a number of minute co-ordinated studies on cases admitted into the Tropical Ward of the Royal Southern Hospital—material which, though it offered peculiar facilities for research, had long remained neglected owing to want of funds. The cases (occurring in the clinics of Dr. Macalister, Dr. Lloyd Roberts, and myself) were placed in charge of Dr. David Thomson; the chemical studies in charge of Dr. G. C. E. Simpson; the parasitological studies in charge of Dr. H. B. Fantham. Parallel researches on animals were also assigned to Dr. John Thomson who is working under Sir Edward Durning-Lawrence's fund for the investigation of the effect of temperature on disease; Dr. V. T. Korke (Research Fellow) has studied coagulation times and other details; the literature was in charge of Mr. W. R. Drawz, the Malaria Bibliographer (Advisory Committee's Fund); and much valuable help has been given by the staffs of the University, the School of Tropical Medicine, and the Royal Southern Hospital of Liverpool, and by Sister Linaker of the Tropical Ward.

The researches were commenced on January 1, 1910. A paper by Dr. David Thomson and myself, describing a regular periodical increase of the trypanosomes in a case of Sleeping Sickness, was published;* and we now present to the Society brief accounts of our results regarding malaria, blackwater fever, trypanosomiasis, and various therapeutical agencies, obtained (mostly by new methods) up to the end of July, 1910. Further details will be published, if necessary, in the 'Annals of Tropical Medicine,' Liverpool.

1. *Preliminary.*—For many years past little information which is both new and exact has been added to our knowledge of the pathology of malaria. This has probably been due to the exhaustion of the older methods of research, which, being purely qualitative, have failed to indicate the precise correlations between the numbers of the parasites present in a patient and the various pathological and therapeutical reactions. For example, out of

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* 'Roy. Soc. Proc.,' B, July 21, 1910, vol. 82.

51 and 38 successful inoculations of men by means of infected blood and infected Anophelines respectively, in not a single one has any exact estimate been given of the numbers of parasites inoculated, or recovered after the lapse of the incubation period; and, though many researches on quinine have been made, we know of none in which its direct effect upon the numbers of the parasites in the patient has been correctly measured. Moreover, the older methods often failed to reveal the parasites at all, unless they were present in large numbers. Hence our first care was to elaborate more exact methods both for detection and for enumeration. This was done early in the year (by R. R. and D. T.)—though we have by no means reached finality yet. The new methods were next employed for all the cases in the Tropical Ward, daily counts of the parasites and often of the leucocytes, together with other estimations, being made (by D. T.). At the same time parallel chemical studies were carried out (by G. C. E. S.) and therapeutical ones (by D. T.); and the measurements were carefully charted and compared. Even at this preliminary stage the results (which are given at the end of this paper) include more accurate verifications of some old conjectures, and demonstrations of some new theorems.

2. *The Detective Method Used.*—In the ordinary methods the blood is spread out in a thin film, liquid or dry—1 cu. mm. covering, say, 4 sq. cm. of area. If the field of the oil-immersion lens has a diameter of 0·165 mm., and can be thoroughly searched by the eye in three seconds, about 12 hours' work would be required to examine the whole of 1 cu. mm. of blood in this way; and if the specimen contains only one parasite it may not, by bad luck, be found until nearly the whole of the area has been searched. We have, therefore, employed the "thick film process" described by one of us (R. R.) in 1903. In this 1 cu. mm. of blood is spread out over only about one quarter of a square centimetre or less, and dried; the hæmoglobin is then gently washed out with water; and the residue, consisting of parasites, leucocytes, platelets, and the stromata of the red corpuscles, is carefully stained by any convenient method, with or without fixation. By this simple means the whole of 1 cu. mm. of blood can be searched in less than an hour; but the identification of the Plasmodia requires considerable practice.

3. *The Enumerative Method Used.*—The younger Plasmodia are too small to be counted by an ordinary hæmocytometer; hence their number has usually been estimated by comparison with the number of red or white cells, the latter figure being determined by the hæmocytometer. This gives a large compound error; for if e is the percentage error made in counting the red or white cells in one specimen, and e' is the percentage error made in

computing the proportion of parasites to each corpuscle in that specimen, then, by a simple calculation, the total percentage error will be $e+e'+ee'/100$. (For example, if $e = +5$ per cent. and $e' = +10$ per cent., the total percentage error will be $+15{\cdot}5$ per cent.)

Our method consists simply in making a measured quantity of blood into a thick film preparation and then counting *all* the parasites in it. The blood is measured by determining the diameter of a fine capillary tube under the microscope and then calculating and marking off the length of the tube required to hold the given quantity (as suggested by Dr. Wakelin Barratt). Thus a uniform tube of 0·180 mm. diameter and 4 cm. length will contain 1·018 cu. mm.—a convenient size. The blood is sucked up into the tube and then quickly discharged upon the glass slide in one or several measured droplets, first samples being rejected. The dehæmoglobinised thick film is fixed before staining, in order to avoid loss, and the whole area is scrupulously searched with the aid of a sliding stage.

Mr. M. Greenwood, Junior, informs us that if n is the number of parasites counted in the unit (say, 1 cu. mm.) of blood, and m is the number of such units in the patient's whole body, then the total number of parasites in him, assuming uniform distribution, will be $mn \pm 0{\cdot}67449m\sqrt{n}$. Thus the probable percentage error is $67{\cdot}449/\sqrt{n}$, the factor m cancelling out from the ratio. This is important, because it shows that the error depends, not on the magnitude of the measured sample taken, but upon the number of parasites actually counted in it. Hence, in order to obtain results within an assigned error, we take a large sample when the parasites are scarce and a small one when they are numerous; the number of parasites which we must find and count being given by the formula $n = 4550/e^2$, where e is the permissible error.

The greatest error occurs when the parasites are so scarce that it is difficult to find enough of them to count up to the required standard; or when they are so numerous that it is difficult to measure a volume of blood small enough to contain an easily countable number of them. In these preliminary researches we have seldom examined at one sitting more than 1 cu. mm. of blood, or less than $\frac{1}{4}$ cu. mm.

When the parasites were very numerous, we have sometimes fallen back on estimating their number by comparison with leucocytes, but are now elaborating improvements and special instruments for such, and other, details. The method is also accurate and rapid for other blood parasites, and for leucocytes. Other sources of error are (1) inaccurate measurements of the quantity of blood used, and (2) difficulty of seeing or distinguishing badly stained parasites.

One kilogramme of blood of average density (1057·5) contains 945,626 cu. mm., and the blood in a man's body is estimated to weigh about 4·9 per cent. of the total body weight—from which data it is easy to compute the total number of parasites in a patient. A man of 64·74 kilogrammes, or about 10 stone, will contain about 3,000,000 cu. mm. of blood.

4. *The Cases studied* by us numbered 33. All had been infected in West Africa or America, so that none was of less than some weeks' duration when admitted into hospital in Liverpool. All were males of from 18 to 60 years of age. They were mostly shipmen or traders, and two were negroes. The parasites of all were counted almost every day and sometimes several times a day; and the temperature was taken every four hours, or more often. One case showed *Plasmodium malariæ*, together with crescents (sexual forms of *P. falciparum*). Eight showed *P. vivax* only, and 24 *P. falciparum* only, two of the latter containing only sexual forms. Quinine was often withheld for some days while other methods of treatment were used. The cases were studied for over 600 days altogether. There were no deaths.

All the cases will be recorded in the 'Annals of Tropical Medicine,' vol. 4, No. 3, but charts of Cases 17 and 20 are given on pp. 164 and 165.

At this preliminary stage of the enquiry we have undertaken to study only the gross daily correlations between the numbers of parasites and other phenomena—leaving more minute hourly or four-hourly analysis to future work. Hence, if a number of counts or other observations have been made on one day, we have recorded only the averages in the tables. For temperatures, however, it has been thought best to record maxima instead of averages, and, as the tables should be as economical as possible, we have used the *hæmatothermic scale* suggested by one of us (R. R.), in which the 10° between 95° and 105° F., or between 35° and 45° C., are divided into 100 parts. Except in Case 6, specially studied by Dr. Fantham, we have not distinguished in the tables the various forms, sets, or stages of *P. vivax*—this being reserved for future study. Similarly, the treatment, leucocytes, hæmoglobin, and urobilin are not differentiated too minutely. Case 25 was counted by Dr. Korke.

5. *The Correlation between the Parasites and the Fever.*—It is well known that fever is caused only by the asexual forms of the parasites, but though these are generally thought to be more abundant during pyrexial periods, no extensive numerical studies on the point appear to have been made, with the result that some sceptics still profess doubts on the subject. Our cases, except four without fever (Cases 1, 15, 16, 27), would seem to indicate a very strong (almost convincing) correlation. Twenty-one of them suffered each from one pyrexial period, lasting from one to seven days, and preceded

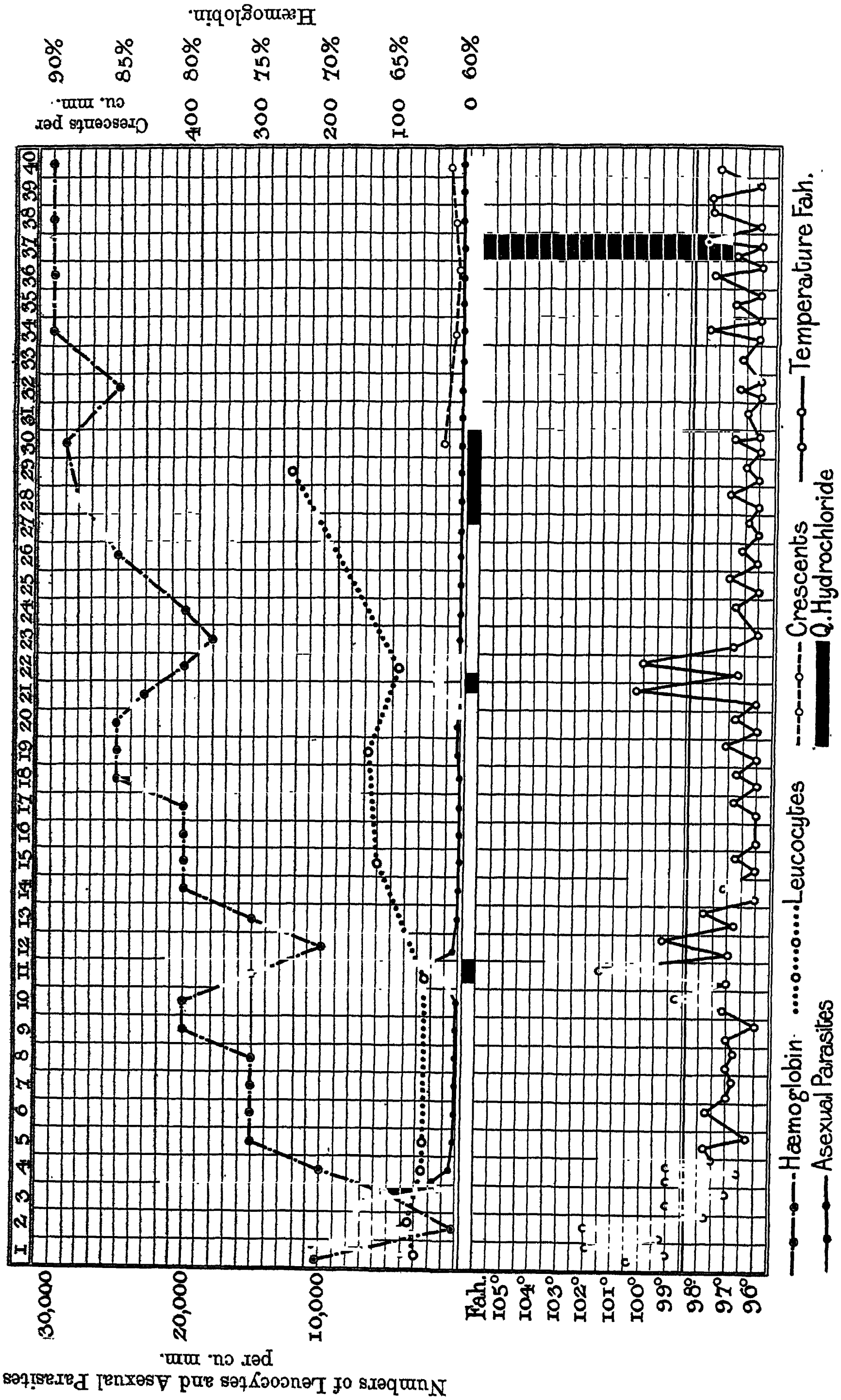
or followed by apyrexia, and in all, except Case 32, the asexual parasites were very much more numerous during the pyrexial period. Nine of the cases (7, 17, 18, 19, 20, 23, 24, 26, 32) suffered from one or two relapses each (13 relapses altogether), and every relapse, as well as every original attack, was associated with a comparatively large number of parasites (43 pyrexial periods altogether). Similarly, there were 46 apyrexial periods (including the four cases without fever), all associated with comparatively small numbers of asexual parasites, and conversely not a single marked rise in the number of asexual parasites took place without corresponding fever.

For further detail we compare the numbers of parasites per cubic millimetre counted on days of fever with those counted on days without fever. A febrile day is taken as any one on which the patient's temperature exceeds 98°·6 F. (= 36° hæmatometric scale), or any one which comes between two tertian paroxysms (Cases 9, 14, 24). The non-febrile days on which no asexual forms could be found in 1 cu. mm. of blood are omitted. The results are—

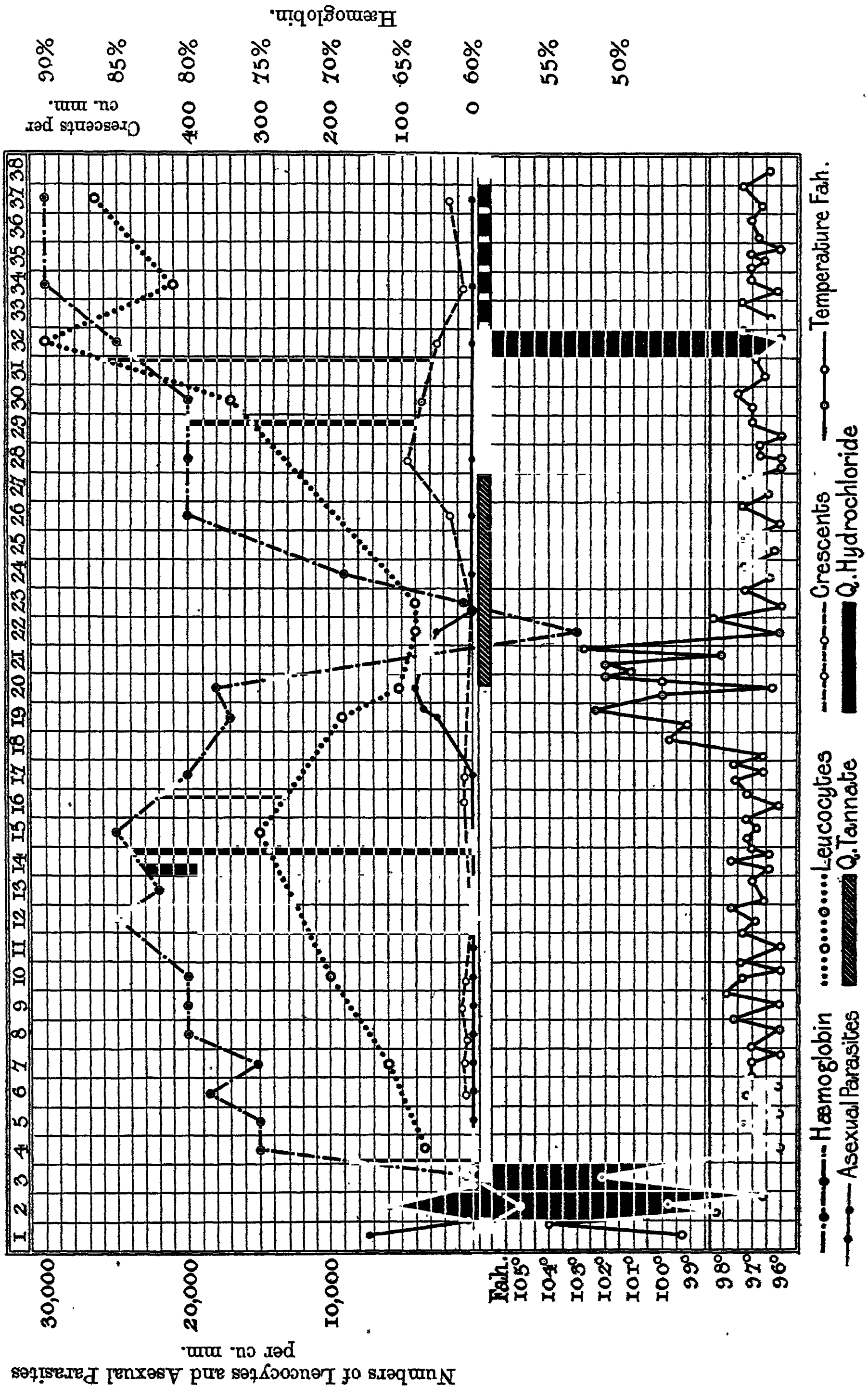
	Cases.	Days.	Total parasites.	Average per day.	Ratio.
			P. vivax.		
Fever	8	30	136,062	4,535	36·3
No fever	8	68	8,487	125	
			P. falciparum.		
Fever	21	97	1,319,880	13,607	29·6
No fever	19	83	38,277	461	

If we had not omitted the non-febrile days on which no parasites were found, the non-febrile daily averages would have been much smaller than the figures given above, and the ratio of the febrile averages to them much larger, so that the febrile excess is very marked. An examination of the details will further convince the reader of the great fall in temperature which accompanies the fall in the number of parasites. We should note that the comparatively small numbers of *P. vivax* found are due to the fact that the specimens generally contained a large proportion of mature parasites, not as yet disintegrated into spores, these of course being absent from peripheral blood containing *P. falciparum*, in which only spores or young forms generally occur. There is no reason for considering quinine in this connection, because it probably affects the fever only through its action on the parasites.

CASE 17.—R.B. *P. falciparum.*



Case 20.—F.B. *P. falciparum.*



6. *The Degree of Fever Associated with Various Numbers of Parasites.*—We know of no extensive and exact studies on this point, and ours are not yet complete. On 47 occasions the number of parasites was carefully compared with the maximum degree of temperature reached during the febrile paroxysm caused by them, and the following results were obtained:—

P. vivax (12 occasions).

No. of parasites	1500	1400	580	440	430	260	256
Maximum temp., deg. H.F.	84	101	97	102	76	76	54
No. of parasites	232	150	133	100	83		
Maximum temp., deg. H.F.	46	44	34	34	20 (rigor)		

(Coefficient of correlation), $r = 0 \cdot 68561 \pm 0 \cdot 10318$.

P. falciparum (35 occasions).

No. of parasites	303,000	58,000	55,000	54,000	50,000	45,000
Maximum temp., deg. H.F.	108	82	88	100	98	76
No. of parasites	36,000	34,000	31,000	26,000	26,000	25,000
Maximum temp., deg. H.F.	60	86	76	94	101	80
No. of parasites	16,000	15,000	15,000	15,000	9,700	9,000
Maximum temp., deg. H.F.	52	54	54	70	56	70
No. of parasites	7,500	7,400	6,700	6,500	6,000	5,800
Maximum temp., deg. H.F.	90	40	86	83	52	70
No. of parasites	5,200	4,000	4,000	3,600	3,000	2,600
Maximum temp., deg. H.F.	76	70	40	80	38	64
No. of parasites	2,500	2,000	1,860	1,000	200	
Maximum temp., deg. H.F.	50	46	38	15	50	

(Coefficient of correlation), $r = 0 \cdot 60331 \pm 0 \cdot 07251$.

These figures, together with the numerous ones given in the table of cases, show a very marked correlation, but there are, of course, many minor deviations. In numbers of the specimens there were certainly two sets of parasites of different ages, which were often counted together, though only one set could have been concerned with the fever. It is probable also, though by no means certain, that the resistance to the toxin of the Plasmodia varies, not only in different persons, but in the same person at different stages in the course of his infection and under different physiological conditions. Our new methods will, we hope, enable us shortly to undertake very detailed studies on these points.

7. *The Pyrogenic Limit.*—It is quite evident that if the parasites fall below a certain limit, they are no longer numerous enough to cause fever. Thus with *P. vivax*, an average of 125 parasites of all ages and forms counted during 68 days in 8 cases, and with *P. falciparum* an average of 460 asexual forms counted during 83 days in 19 cases, failed to produce fever. The actual limit above which they become pyrogenic probably varies in different cases. With *P. vivax*, as many as 1500, 852, and 540 were found in Cases 2, 6, and 7

without fever, and as few as 150 and 50 in Cases 5 and 9 with very slight fever (98°·6 F.), so that about 200 to 500 may perhaps be taken as the usual limit. With *P. falciparum*, as many as 1620 (asexual) were found as an average of four non-febrile days in Case 26, and 1196 as an average of six non-febrile days in Case 25; while in Case 18 from 16 to 1860 were present for 22 days without causing any marked fever, so that 600 to 1500 may perhaps be adopted as the usual limit. With *P. malariæ* (Case 1), an average of 79 were present for 13 days without fever, but about 140 twice caused slight rigor.

8. *The Asexual Forms between the Relapses.*—It is generally thought and taught that these forms tend to "disappear" between the relapses—though of course exceptions, especially in children, are noted—and to reappear during them; and several hypotheses, such as that of parthenogenesis (F. Schaudinn), have been advanced to explain the supposed phenomenon, and have led to what appears to be much waste of work. We find that the asexual forms do not necessarily disappear at all between the relapses, but are generally still to be found in small numbers per cubic millimetre on most of the days. The apyretic periods preceding the 13 relapses in our cases (7, 17, 18, 19, 20, 23, 24, 26, 32) lasted 114 days altogether (nearly nine days each on the average). On 18 of these days no observations were made; but on the remaining 96 days the parasites were found in small numbers on 59, or 61 per cent., and were not found only on 37. Moreover, the general trend of the curves suggests that they were not found on these days only because their numbers were a little too few for detection. The parasite curve, at its height during a pyrexial period, generally falls very rapidly at first and more slowly later, and tends to reach its lowest about half-way between two apyrexial periods. At this point it may or may not remain above the detectable limit (by thick-film methods). After this it was observed (by D. T.), especially in Cases 7, 17, 23, and 24, to begin mounting slowly at first, until, when it reached the pyrogenic limit, another pyrexial period commenced. All this is scarcely compatible with the speculation that the apyrexial periods are due to the *abrupt* death of most of the asexual Plasmodia, or to their conversion into "resting stages," etc. Nor do such speculations appear to be at all necessary. It is easy to see that the survival of comparatively small numbers of the asexual forms will suffice to keep the infection alive, not only for the short periods observed by us, but for "relapses of long interval," and for months or years. Obviously, if only a few parasites per cubic millimetre are present, they may easily be overlooked in the small amount of blood (say 0·1 cu. mm.) usually examined by thin film, and may then be reported as being absent: yet many millions may still exist

in a patient who contains, say, 3,000,000 cu. mm. of blood. The speculation regarding parthenogenesis in malaria, and the case of Schaudinn supposed to support it, have been criticised elsewhere by one of us (R. R.).* In two of our relapsing cases (19, 26), no sexual forms at all were found during 35 different thick-film examinations. Even if they were present in small numbers (crescents do not collect in the spleen), yet the numbers of asexual parasites found in or just before the relapses (24 and 400 per cu. mm.) cannot be explained by parthenogenesis unless we suppose that each sexual form produced by "gametoschizogony" 840 or 14,000 spores! Until better evidence for such views is adduced, our results justify the doctrine that the malarial infection is kept continuously alive simply by the persistence of the asexual forms in varying numbers, and that fever occurs only when the forms are numerous enough to produce it.

9. *The Effect of Quinine on the Asexual Forms.*—The destructive effect of the drug is, of course, everywhere recognised, though very little completely satisfactory statistical evidence can be cited in proof. Many laborious researches have been made regarding the comparative utility of the various salts, but these have been confined almost entirely to estimating the rate and percentage of absorption, judged from urinary elimination. The subject is of the greatest sanitary and medical importance; but, so far as we can see, it can be usefully studied only by the more detailed enumerative analysis which we propose soon to undertake. Our daily analysis gives a little more light; but the following figures may be mentioned. Cases 15 and 16 showed no asexual forms; Cases 11 and 13 were so severe that quinine was given at once; Cases 1, 5, 27, 31 and 32 were so mild that quinine was withheld during the period of observation; and in the remaining cases the drug was withheld only for some days (except an occasional necessary dose), and was then given continuously while the parasites were still being counted. We have therefore added together all the daily counts during the periods of no-quinine and quinine respectively. With eight cases of *P. vivax* there were 45 no-quinine days, showing 104,032 total parasites, or an average of 2,312 a day; and there were 74 quinine days, with 31,769 total parasites, or an average of only 429 a day (one-fifth). With *P. falciparum* (19 cases), 147 no-quinine days showed an average of 3,136 asexual forms, and 148 quinine days an average of 1,535 (one-half). We should have expected that the proportion of quinine-day parasites would have been smaller; but the no-quinine counts are diminished by the inclusion of the five mild cases and also by doses of 10 to 20 grains which were given on 10 isolated occasions. On quinine days the doses were generally

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* 'Prevention of Malaria' (Murray, 1910).

20 to 30 grains a day, and Cases 11, 13, 14, 15 and 16, and parts of other cases are omitted because they were unsuitable. Great falls in the number of parasites occurred, even without quinine, in Cases 5, 17 and 26; while, on the other hand, small numbers of asexual forms remained in some cases for five or more days in spite of considerable daily dosage. In Case 23 a severe relapse began six days after the stoppage of quinine tannate, which had been given for nine previous days in 10 to 30 grain doses daily. No other relapses occurred after continuous quinine treatment; but 12 occurred without it.

10. *Observations on the Sexual Forms.*—It is, of course, generally held—and probably quite rightly—that these are developed (by a cytological process not yet clearly seen) from the asexual forms. But, at the same time, no one has been able to note any correspondence between their numbers—many of the former may be present when the latter are very scarce, and *vice versâ.* This rule, which is fully confirmed by our figures, has always been difficult to reconcile with the accepted theory of origin. On comparing our curves, however, the remarkable fact was observed (by D. T.) that there often seems to be indeed a correspondence, but that it is delayed for from 8 to 10 days—that is, that the sexual curve tends to rise that number of days after a rise in the asexual curve. Out of our 25 infections with *P. falciparum* 18 showed crescents, and of these Cases 14, 22, 17, 18, 20, 23, 24 and 30 suggest this phenomena, while in 17 and 20, the rises are sufficiently isolated to show it most distinctly. In Case 17, asexual rises existed on the 1st to 3rd days, the 11th to 12th and the 21st to 22nd days, separated by apyrexial periods during which few asexual and no sexual forms were found; but on the 30th day (eight days after the last relapse) crescents appeared for the first time. In Case 20, an asexual relapse commenced on the 17th day and reached its height on the 20th day. A very few crescents had been found all the time; but a marked rise in their numbers commenced on the 26th day and reached its height on the 28th day. The most ready explanation is that the *crescents require* 8 *to* 10 *days for development,* during which they remain undetected, probably in the internal organs of the host. This is confirmed by the fact that numerous small ones are seen at the commencement of a rise; and also by the observation (frequently made) that in fresh infections the crescents seldom appear until a week or more after the first attack of fever. Strong confirmation is also given by Case 6, in which Dr. Fantham noted a sudden appearance of sexual forms (*P. vivax*) on the 11th day, 7 to 10 days after the original asexual rise, and after the patient had been taking 30 grains of quinine daily for a week, suggesting that the same phenomenon holds for *P. vivax* also.

Other observations are as follows:—(1) By no means every asexual rise is followed by a sexual one. In seven cases (13, 19, 25, 26, 28, 31, 33), no crescents at all were seen. In Case 13, a negro, 173,400 asexual forms per cubic millimetre failed to produce any; and no crescents were observed in another negro (Case 28). (2) The number of crescents never exceeded 5 per cent. of asexual forms found in the generation which (hypothetically) produced them, and were generally in a much smaller proportion. (3) We could find no relation to youth, vigour, amount of hæmoglobin, duration of infection (from 3 to 30 weeks), or season of first infection. (4) The daily counts of crescents generally show marked daily variations, but we think that after considerable doses of quinine have been given for some days these daily variations tend to be smoothed out (Cases 16, 18, 22, 24). (5) In Cases 15, 14 and 16 particularly, a distinct tertian tendency was observed in the daily variations of crescents. This appears not to have been previously noticed, but is just what would be expected in the case of a tertian parasite; though, of course, the curve would be confused in a double tertian. (6) Sometimes the crescent curve suddenly rises with great rapidity, reaches its maximum, and then begins to fall on the next day with equal rapidity; though, later, the fall often tends to be much more slow (Cases 14, 15, 22, 23), especially when the numbers are very small. Apparently, the greater the maximum the quicker the fall (Cases 14, 15); and the fall is sometimes jagged, with a tertian tendency (*ibid.*). (7) Sometimes the maximum is irregularly maintained for a number of days (Cases 16, 18, 24), especially when quinine has not been previously given. (8) The crescents remained detectable for as many as 31, 32, 32, 35 and 44 days in Cases 20, 22, 23, 24 and 18 respectively; and finally disappeared (in 1 cu. mm. of blood) in Cases 1, 11, 12, 16, 21 and 23.

From these data we think (1) that a varying percentage of asexual forms are constantly generating crescents, which, after about 8 to 10 days, appear in the peripheral blood. Hence, when the asexual forms remain sufficiently numerous for a period, as when no quinine is given, the stock of crescents is being constantly replenished. Thus the mortality among the older crescents is constantly being compensated for by new arrivals, and the total numbers appear to remain constant; an appearance which has given rise to the generally accepted hypothesis (perhaps erroneous) that the crescents survive for weeks. On the other hand, the sudden rise and fall of their numbers seen in Cases 14, 15, 22 and 23 suggest that they really survive only for a short time after their appearance in the peripheral blood. In Cases 16 and 24, however, a few crescents were still found after quinine had been given continuously for 16 to 18 days, suggesting that some of them may be able to survive for longer periods. In Cases 20, 22 and 23, they appeared in increasing numbers for 4 to 6 days after continuous 30-grain doses of quinine had been commenced, suggesting that the drug has no effect upon them when they have once been generated. But quinine appears to affect the numbers of crescents indirectly by cutting off the source of supply. Though many researches upon the effect of quinine on crescents (a subject of the greatest sanitary importance) have been attempted, so far as we know, the possibility of a constantly continued supply has not previously been taken into consideration. We are continuing researches on the point.

11. *The Leucocytes* were counted frequently, but not always daily; at first by the hæmocytometer, but in the later 20 cases by the thick-film method. The latter method has the advantages of putting the leucocytes closer together and of enabling us to estimate them at the same time with the parasites; but, like the hæmocytometer, it is not very appropriate for differential counts, because of occasional uncertainty in distinguishing the various kinds. Accordingly, these were made (in six cases) by thin film, 500 cells being examined on each occasion.

Our results are, as shown in all the cases, that during the pyrexial periods the total leucocytes are comparatively few, being frequently as low as 2000 to 3000 per cubic millimetre (Cases 17, 18, 23, 24, etc.). But as the asexual forms and the fever diminish, the total leucocyte curve rises, and about seven days later exceeds the normal, and may reach 20,000 and even, rarely, 30,000 per cubic millimetre (Case 7)—though this height is not maintained. The very high counts seem to be associated with much quinine. We observed generally that the so-called polymorphonuclear leucocytes, though rather few during the pyrexial periods, do not vary much from day to day, but about 7 days after the fever has abated their numbers are markedly increased and vary much. The so-called mononuclear percentage was always in excess throughout the cases, even long after the disappearance of all forms of the parasites—for six weeks afterwards in Case 1, though quinine had been given daily during that period (out of hospital). We think, therefore, that a high mononuclear percentage is likely to be always of value in diagnosis in the absence of parasites. The normal ratio of total mononuclears to polymorphonuclears is about 35 per cent., but in our cases it was often 60 per cent, and in Case 30 reached 80 per cent. It tends to fall slightly with great improvement in health. During the individual daily paroxysms, as shown by Stephens and Christophers, the total mononuclears are rather low during the height of the fever, but show a marked increase during the following remission. This increase is chiefly due to the large mononuclears. This process is repeated with each paroxysm, and if a paroxysm fails the mononuclears remain (Case 9 especially). But we could not find these variations in the absence of fever and many parasites. This mononuclear reaction, which we have also seen in our case of trypanosomiasis, appears to be exactly comparable to the polymorphonuclear reaction described by F. W. Andrewes* as generally occurring in bacterial diseases.

12. *Various Therapeutic Agents.*—Methylene blue was tried in three cases in 12-grain daily doses; the parasites diminished, but we cannot draw any conclusions from the figures. In Case 24, altogether 36 grains of soamine were injected intramuscularly in 5 to 10 grain doses on five occasions during 12 days, and yet a smart relapse occurred during and after the last dose. In the same case, just before the same relapse, X-rays had been applied over the spleen and abdomen for 20 minutes. The asexual parasite rise had commenced before the application. In Case 24, faradic and galvanic currents were applied over the spleen, each for 10 minutes, but crescent counts made immediately before and two hours after the application remained unaltered.

13. *Hœmoglobin.*—This was estimated in most of the later cases. The percentage always fell during fever, and began to rise rapidly shortly afterwards, proving the destructive effect of the parasites and the rapid recuperation of the patients. The greatest fall was 25 per cent. after four days' fever (Case 20); but more detailed work is required to trace correspondence with the number of parasites. The lowest percentage found was 53 per cent. All the patients were anæmic at first, but the hæmoglobin rose rapidly with improvement of health, and very rapidly in the more vigorous subjects. We decided not to attempt counts of red corpuscles, as these have been made so frequently already. The elimination of the hæmoglobin and the findings in a case of blackwater fever will be dealt with in the following paper by Dr. Simpson and in one by ourselves.†

14. *Summary.*—(1) There is a very decided correlation between the number of asexual Plasmodia found in the peripheral blood and the fever.

(2) As a rule, no fever exists unless the asexual forms exceed some hundreds per cubic millimetre.

(3) The asexual forms do not always disappear between relapses (as often thought) but tend to persist in small numbers per cubic millimetre, and often increase again for some days before the actual febrile relapse occurs.

(4) These observations give a coherent theory of the malarial invasion, according to which the infection is kept alive indefinitely by the ordinary sporulation of the asexual forms, and not by parthenogenesis or by resistant

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* 'Lancet,' June, July, 1910.

† 'Annals of Tropical Medicine,' Liverpool, vol. 4, No. 3.

forms; and fever recurs only when the parasites are numerous enough to produce it.

(5) We estimate from our cases that considerable continued doses of quinine reduced the asexual forms by 50 to 80 per cent.

(6) There are strong reasons for supposing that the sexual forms require eight to ten days for development; that the often noticed long persistence of crescents is not due to their long life (as generally thought) but to constant replenishment of the stock by fresh broods; that they sometimes show a distinct tertian periodicity; and that quinine does not affect them when once generated, but ultimately reduces their numbers by destroying the generating cells. The sexual forms were never seen to produce fever.

(7) The leucocytes are below normal during febrile periods and above normal afterwards. The percentage of mononuclears rises greatly after paroxysms, and is always in excess of the normal.

(8) Methylene blue, soamine, X-rays, and faradic and galvanic currents had no results in a few experiments.

(9) The hæmoglobin falls markedly with fever, but rises rapidly with convalescence.

(10) The fæcal urobilin shows marked correlation with the occurrence of fever, and is specially studied in the accompanying paper by Dr. G. C. E. Simpson.*

Many other deductions may be based on our observations, but will be better considered after more detailed counts have been made.

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* 'Roy. Soc. Proc.,' B, vol. 83, p. 174.

On Hæmoglobin Metabolism in Malarial Fever. (Preliminary Note.)

By G. C. E. Simpson, B.A., B.Sc., F.R.C.S.

(Communicated by Major R. Ross, F.R.S. Received October 12,—Read December 8, 1910.)

The object of this study was to examine some of the cases of malaria reported upon by Major R. Ross and Dr. David Thomson in their paper on "Enumerative Studies on Malarial Fever," with especial reference to the meaning of the fall of hæmoglobin there recorded during pyrexia, in the ultimate hope that some light may thus be thrown on various problems connected with malaria, and especially with blackwater fever.

The chief product of hæmoglobin metabolism, and the only one available for quantitative work, is the urobilin which is removed from the body partly in the urine, partly in the fæces. Previous observers have devoted much attention to the quantitative determination of urinary urobilin in health and disease, and have shown that it is increased in diseases connected with pyrexia and with hæmolysis. In benign tertian malaria the urinary urobilin is excreted in quantities comparable to those in other diseases. In malignant tertian malaria the excretion of urobilin in the urine is somewhat higher. Occasionally very marked urobilinuria occurs.

The main channel of elimination of urobilin, however, is the fæces, and this fact has been somewhat neglected in comparison with the urinary estimations. Serious difficulties are met with owing to variations in composition of fæcal urobilin. The amount of urobilin in the fæces nearly always completely overshadows that in the urine. In our cases of benign tertian malaria, the excretion of fæcal urobilin is again found to be comparable to that in other pyrexial diseases. In malignant tertian malaria, however, a high output is the rule, and occasionally a very large output occurs. This is of importance as showing that great hæmolysis has occurred and that an amount of hæmoglobin (25 per cent.) has been destroyed (and its products excreted) comparable to the fall recorded by the hæmoglobinometer; this shows that the hæmoglobinometer readings denote an actual destruction of corpuscles, and not merely a relative fall in the peripheral circulation. The number of corpuscles destroyed is much larger than the number infected by parasites.

The eliminating organs deal with a large amount of hæmoglobin during the pyrexial periods and the days which immediately follow; this would seem to indicate a continual loading of the blood plasma with freed hæmoglobin; but the amount of hæmoglobinæmia is kept below that necessary to produce hæmoglobinuria by the continuous activity of the liver.

In a case of blackwater fever examined by Major Ross, Dr. Thomson, and myself, the fæcal excretion of urobilin reached an astonishing figure, representing practically the elimination of an amount of hæmoglobin equal to the whole of the circulating blood. This was accompanied by slight urobilinuria and very slight hæmoglobinuria, showing that the hæmoglobinuria of blackwater fever is apparently only an overflow from the normal channel of excretion, and that the hæmolysis has reached an exceptional figure. Further studies are being made on the subject.

Colour-Blindness and the Trichromatic Theory of Colour Vision.
Part II.—Incomplete Red or Green Blindness.

By Sir W. de W. Abney, K.C.B., F.R.S.

[This paper is published in Series A, vol. 84, No. 572.]

On the Sensibility of the Eye to Variations of Wave-length in the Yellow Region of the Spectrum.

By Lord Rayleigh, O.M., F.R.S.

[This paper is published in Series A, vol. 84, No. 572.]

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*Trypanosome Diseases of Domestic Animals in Uganda.**

IV.—Trypanosoma uniforme, sp. nov.

By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, R.A.M.C.; and Captain F. P. MACKIE, I.M.S. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10).

(Received October 1,—Read December 8, 1910.)

[PLATE 12.]

INTRODUCTION.

The name *Trypanosoma uniforme* has been given to this species on account of the uniformity in shape and general appearance which characterises it.

Only four oxen were found by the Commission to show this trypanosome in their blood. One was received from Sir Apolo Kagwa, K.C.M.G., the Prime Minister, on August 30, 1909, and its blood at once inoculated into a series of animals. The other three came from the Uganda Company's estate at Namukekera.

The ox, goat, and sheep were found to be susceptible, while the monkey, dog, guinea-pig, rat, and mouse proved refractory. This was the case with *Trypanosoma vivax*, and in truth these two species resemble one another very closely. *Trypanosoma uniforme* differs from *T. vivax* in size, and perhaps to a slight extent in shape; but, from the small amount of material at the disposal of the Commission, it would be rash to generalise.

The few facts gathered will, therefore, be put on record, in order to draw the attention of future workers on Uganda trypanosomes to its presence.

MORPHOLOGY OF TRYPANOSOMA UNIFORME.

A. *Living, Unstained.*

This is a small and active trypanosome, which has a marked translatory movement in the field of the microscope. This movement, however, is not to be compared with that of *T. vivax* in point of rapidity or range. The rapid vibratory movement of the body and flagellum sometimes slows down for a perceptible fraction of time; but this trypanosome does not become completely quiescent as is commonly the case with *T. pecorum*. The cell-contents are clear and homogeneous, without any appearance of a vacuole.

B. *Fixed and Stained.*

Length.—This trypanosome is smaller than *T. vivax*. The average is 16·0 as against 23·7 microns. The following table gives the average length of this trypanosome in the ox, goat, and sheep. Twenty trypanosomes are drawn and measured from each preparation, the average length reckoned, and the length of the longest and shortest given :—

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* Continued from 'Roy. Soc. Proc.,' 1910, B, vol. 83, p. 27.

Table I.—*Trypanosoma uniforme.*

No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
1437	Ox	Osmic acid	Giemsa	16·0	17·5	15·0
1442	„	„	„	16·0	18·0	14·0
1442	„	„	„	16·3	19·0	14·0
1581	„	„	„	16·5	19·0	14·0
1732	„	„	„	15·5	17·5	13·5
1734	„	„	„	15·3	17·0	14·0
1689	Goat	„	„	16·4	17·5	15·0
1694	„	„	„	17·7	19·0	16·0
1694	„	„	„	14·7	16·0	13·0
1497	Sheep	„	„	17·8	19·0	16·0
1497	„	„	„	14·7	16·0	12·0
				16·0	19·0	12·0

The following table represents the distribution in respect to length of 200 individuals of this species of trypanosome :—

Table II.—Distribution in respect to Length of 200 individuals of *Trypanosoma uniforme.*

No. of expt.	Microns.								Average, in microns.
	12.	13.	14.	15.	16.	17.	18.	19.	
1437	—	—	—	5	10	5	—	—	16·0
1442	—	—	3	2	9	4	2	—	16·0
1442	—	—	1	6	5	5	1	2	16·3
1581	—	—	1	3	8	2	4	2	16·5
1734	—	—	4	8	6	2	—	—	15·3
1689	—	—	—	3	6	11	—	—	16·4
1694	—	—	—	—	2	5	9	4	17·7
1694	—	1	8	7	4	—	—	—	14·7
1497	—	—	—	—	4	7	8	1	17·8
1497	1	—	7	6	6	—	—	—	14·7
Totals	1	1	24	40	60	41	24	9	
Percentages	0·5	0·5	12·0	20·0	30·0	20·5	12·0	4·5	

Breadth.—Varies from 1·5 to 2·5 microns.

Shape.—This species of trypanosome seems to differ in shape from *T. vivax* in that there is not the marked narrowing or constriction opposite the nucleus. The posterior extremity is rounded or blunt, and in this resembles *T. vivax* (Plate 12).

Contents of Cell.—Resembles *T. vivax* in showing the appearance of clear protoplasm with fine alveolar structure.

Nucleus.—Oval in shape and compact; not, as a rule, situated in a narrowed part of the body or waist, as in *T. vivax.* It also seems to be placed about the centre of the body, and does not take up the whole width of the cell as in the closely allied species.

Micronucleus.—Resembles *T. vivax* in being large, round, and terminal.

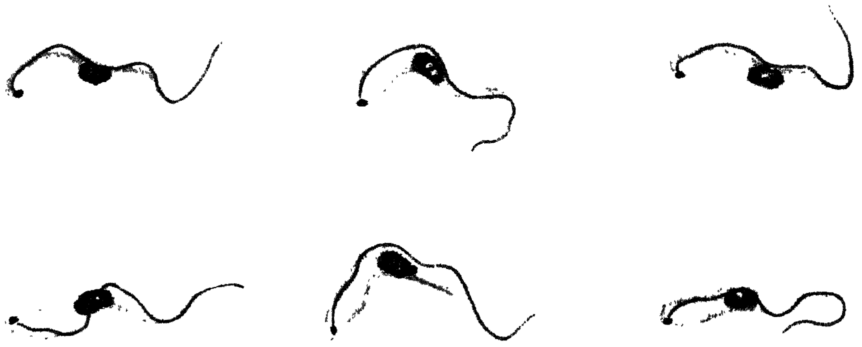
Undulating Membrane.—Narrow and little developed, as in *T. vivax.*

Flagellum.—There is a well-marked flagellum, the free part varying from 2 to 5 microns in length.

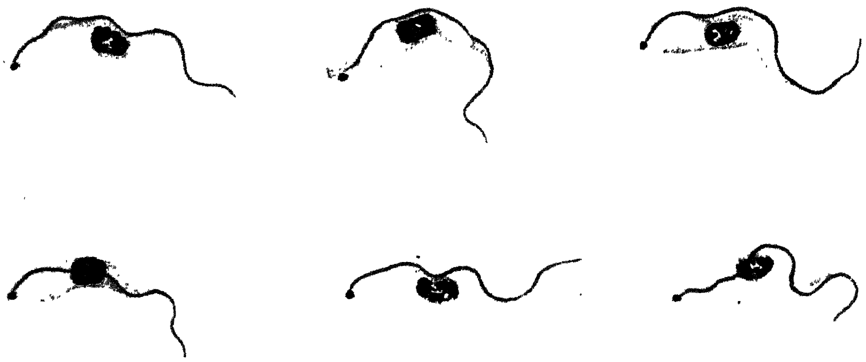
ANIMALS SUSCEPTIBLE TO TRYPANOSOMA UNIFORME.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks
			Cattle.		
1909. Aug. 7	1442	Nat. infec.	?	5	Died of *T. uniforme.*
„ 30	1581	„	?	61	Killed.
„ 11	1732	„	?	79	Died of *T. uniforme.*
„ 11	1734	„	?	80	Killed.
			Goat.		
Sept. 1	1491	Ox 1581	15	18	No *post-mortem.*
„ 16	1689	„	18	34	„ „
„ 18	1694	Goat 1491	9	35	„ „
„ 24	1716	Ox 1581	—	—	Never showed trypanosomes; under observation 40 days.
		Average ...	14	29	
			Sheep.		
Sept. 16	1497	Ox 1581	18	46	Killed.
„ 1	1601	„	—	—	Never showed trypanosomes; died 10 days after inoculation.
„ 24	1717	„	—	—	Never showed trypanosomes; died 13 days after inoculation.
			Monkey.		
Sept. 1	1653	Ox 1581	—	—	Experiment stopped after 34 days.

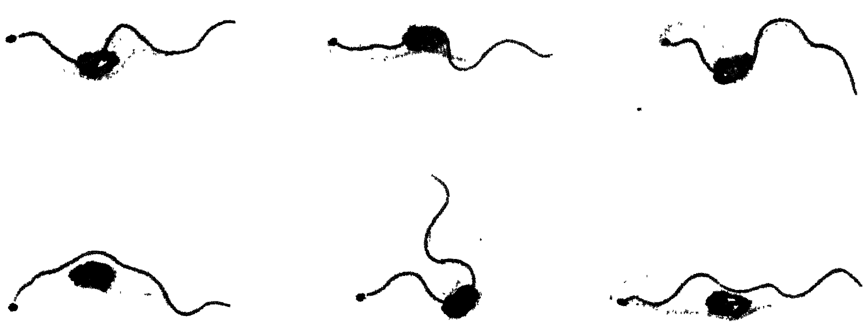
* Duration includes the days of incubation; it dates from the day of infection.



Oxen.



Goats.



Sheep.

M E Bruce, del

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Dog.		
Sept. 1	1600	Ox 1581	—	—	Experiment stopped after 34 days.
			Guinea-pig.		
Sept. 1	1599	Ox 1581	—	—	Experiment stopped after 34 days.
			Rat.		
Sept. 1	1597	Ox 1581	—	—	Experiment stopped after 34 days.
			Mouse.		
Sept. 1	1598	Ox 1581	—	—	Experiment stopped after 34 days.

* Duration includes the days of incubation; it dates from the day of infection.

CULTIVATION OF T. UNIFORME.

No attempt was made to cultivate *T. uniforme.*

CARRIER OF T. UNIFORME.

No experiments were made in the laboratory with *Glossina palpalis* as a carrier of *T. uniforme,* and no evidence is to hand as to what the carrier is.

CONCLUSIONS.

1. *Trypanosoma uniforme* resembles *T. vivax* in shape and general appearance, but differs markedly in size.

2. It also resembles *T. vivax* in not being pathogenic to the smaller laboratory animals.

3. There is no evidence available, as in the case of *T. vivax,* as to what the carrier of *T. uniforme* is.

DESCRIPTION OF PLATE.

PLATE 12.

Trypanosoma uniforme in the ox, goat, and sheep. Fixed osmic acid, stained Giesma. ×2000. Note the large round terminal micronucleus, the oval compact nucleus, situated about the centre of the body, the narrow undulating membrane, and the free flagellum.

*Trypanosome Diseases of Domestic Animals in Uganda.**

V.—Trypanosoma nanum (*Laveran*).

By Colonel Sir David Bruce, C.B., F.R.S., A.M.S.; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, R.A.M.C.; and Captain F. P. Mackie, I.M.S. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10).

(Received October 1,—Read December 8, 1910.)

[Plates 13 and 14.]

INTRODUCTION.

Only two cattle (Experiments 503 and 1118) examined by the Commission at Mpumu were found to harbour this trypanosome in their blood. Both came from the Uganda Company's estate at Namukekera, some fifty miles from Lake Victoria, and both had become infected on the estate.

This species differed from *Trypanosoma pecorum*, in that it did not affect the smaller laboratory animals, such as monkeys, dogs, rats, or mice. Guinea-pigs were also refractory to the disease. Horses, mules, donkeys, and rabbits were not available at Mpumu, so that it is impossible to say what would have been the effect of inoculation in them. Oxen and goats were inoculable, but the only sheep experimented on failed to become infected, although inoculated with the same blood which infected a goat.

It seems rash to recognise *T. nanum* as a Uganda species on the insufficient evidence at our disposal; but yet the fact remains that this trypanosome in every case failed to infect the smaller animals, and, moreover, by placing these cases on record it will draw the attention of future workers to its possible presence.

MORPHOLOGY OF TRYPANOSOMA NANUM.

A. *Living, Unstained.*

T. nanum is indistinguishable from *T. pecorum* in the fresh and living condition.

B. *Fixed and Stained.*

Length.—The following table gives the average length of this trypanosome in the ox and goat. Twenty trypanosomes are drawn and measured from each preparation, the average length reckoned, and the length of the longest and shortest given:—

---

* Continued from preceding paper.

Table I.—*Trypanosoma nanum.*

No. of expt.	Animal.	Method of fixing.	Method of staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
503	Ox	Osmic acid	Giemsa	13·3	15·0	11·0
983	,,	,,	,,	13·1	14·0	12·0
698	Goat	,,	,,	13·9	16·0	13·0
698	,,	,,	,,	15·2	16·0	12·0
882	,,	,,	,,	12·9	15·0	11·0
883	,,	,,	,,	13·3	15·0	11·0
1691	,,	,,	,,	13·7	16·0	12·0
				13·6	16·0	11·0

The average length of *T. nanum* corresponds closely with that of *T. pecorum*, which was 13·3 microns. The question arises as to whether it would not be better for the sake of simplicity to include, for the present at least, this species in that of *T. pecorum*. If the morphology is the same, if the important domestic animals are susceptible to both, if the geographical distribution is the same, and if the carrier is found to be the same, there seems little need of separating the two under different names.

Breadth.—At the widest part from 1·5 to 2·5 microns.

Shape.—Much the same as *T. pecorum*, but perhaps slightly narrower, due to the less development of the undulating membrane (Plate 14).

Contents of Cell.—As a rule homogeneous.

Nucleus.—Oval and situated about the middle of the body.

Micronucleus.—Small and rounded, and situated near the posterior extremity.

Undulating Membrane.—Perhaps a little narrower and simpler than in *T. pecorum*. It was thought to be applied more closely to the body and less thrown into folds than in that species.

Flagellum.—There is no free flagellum.

From a comparison of Tables II and III, Chart 1, and the coloured figures of the two species, it is evident that *T. pecorum* and *T. nanum* resemble each other very closely morphologically.

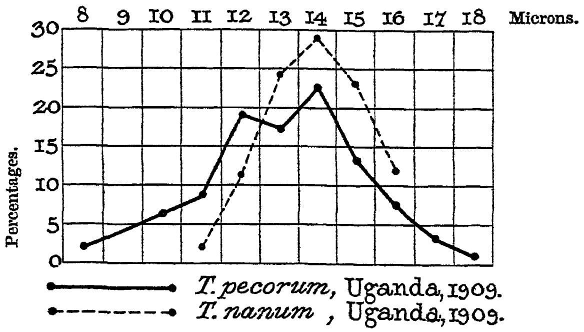
Table II.—Showing the Distribution in respect to Length of 100 Individuals of *T. nanum*, Uganda, 1909.

No. of expt.	Microns.						Average, in microns.
	11.	12.	13.	14.	15.	16.	
689	—	—	7	8	4	1	13·9
698	—	1	—	2	7	10	15·2
882	1	5	10	3	1	—	12·9
883	1	4	5	7	3	—	13·3
1691	—	1	2	8	8	1	13·7
Totals.........	2	11	24	28	23	12	
Percentages	2·0	11·0	24·0	28·0	23·0	12·0	

Table III.—Showing the Distribution in respect to Length of 260 Individuals of *T. pecorum*, Uganda, 1909.

No. of expt.	Microns.											Average, in microns.
	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	
82	—	—	2	6	8	3	1	—	—	—	—	11·7
505	—	—	2	2	9	4	1	2	—	—	—	12·3
—	—	—	—	2	3	6	6	3	—	—	—	13·2
593	—	—	1	—	2	1	3	7	5	1	—	14·5
44	—	—	4	2	5	3	6	—	—	—	—	12·2
559	—	—	—	2	9	4	4	—	1	—	—	12·7
461	—	—	—	—	—	5	4	1	2	6	2	15·3
543	—	—	—	2	—	2	6	6	4	—	—	14·3
1406	—	—	—	1	5	2	5	5	2	—	—	13·7
551	1	—	3	3	3	3	3	2	1	—	1	12·6
626	—	—	3	2	3	2	7	3	—	—	—	12·8
398	—	—	—	—	—	1	9	6	4	—	—	14·6
398	—	—	1	1	3	9	4	—	1	1	—	13·1
Totals ...	1	—	16	23	50	45	59	35	20	8	3	
Percentages	0·4	—	6·1	8·8	19·2	17·3	22·7	13·5	7·7	3·1	1·2	

CHART 1.—Giving Curves representing the Distribution, by Percentages, in respect to Length of *T. pecorum*, Uganda, 1909, and *T. nanum*, Uganda, 1909.



COMPARISON OF T. NANUM, UGANDA, 1909, WITH T. NANUM, SUDAN, 1904.

Thanks to Dr. Andrew Balfour, the Director of the Wellcome Research Laboratories, Khartoum, the Commission is enabled to compare the Uganda strain with the original Sudan strain.

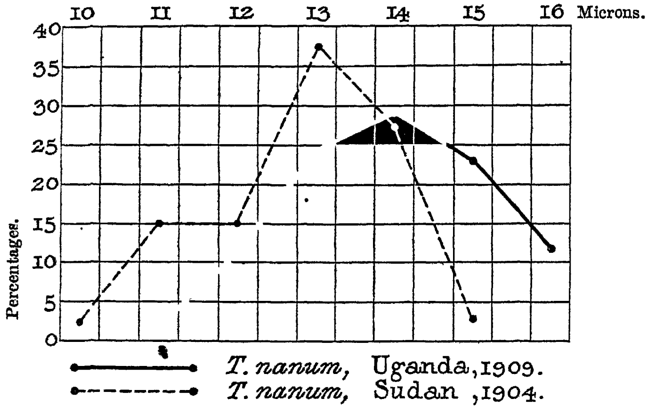
Table IV.—Measurements of Dr. Balfour's Sudan Strain of *T. nanum*, 1904.

Animal.	Method of fixing and staining.	In microns.		
		Average length.	Maximum length.	Minimum length.
Ox	?	12·9	15·0	11·0
"	?	12·7	14·0	10·0

Table V.—Showing the Distribution in respect to Length of 40 Individuals of *T. nanum*, Sudan, 1904.

Animal.	Microns.						Average, in microns.
	10.	11.	12.	13.	14.	15.	
Ox	—	3	4	6	6	1	12·9
Ox	1	3	2	9	5	—	12·7
Totals	1	6	6	15	11	1	
Percentages	2·5	15·0	15·0	37·5	27·5	2·5	

CHART 2.—Giving Curves representing the Distribution, by Percentages, in respect to Length of *T. nanum*, Uganda, 1909, and Dr. Balfour's *T. nanum*, Sudan, 1904.



From a comparison of these tables and the figures in Plate 13 there can be little doubt as to the identity as regards morphology of *T. nanum*, Uganda, 1909, and Dr. Balfour's *T. nanum*, discovered in the Sudan in 1904:—

ANIMALS SUSCEPTIBLE TO T. NANUM, UGANDA, 1909.

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Cattle.		
1909. Feb. 5	503	Nat. infec.	?	245	From Namukekera. Died of *T. nanum*.
June 25	1118	,,	?	3	From Namukekera. Died of *T. nanum*.
Sept. 29	780	Ox 983	—	—	Never showed trypanosomes; still alive after 47 days.
June 11	982	Ox 503	—	—	Never showed trypanosomes; still alive after 141 days.
,, 11	983	,,	20	141	Killed.
			Goat.		
Apr. 12	698	Ox 503	25	137	Died of *T. nanum*.
May 21	882	,,	26	91	,, ,,
,, 21	883	,,	6	46	,, ,,
Sept. 16	1691	Oxen 503 & 982	8	12	,, ,,
		Average ...	16	71	
			Sheep.		
May 21	880	Ox 503	—	—	Never showed trypanosomes; still alive after 82 days.

* Duration includes the days of incubation; it dates from the day of infection.

ANIMALS SUSCEPTIBLE TO T. NANUM, UGANDA, 1909—*continued.*

Date.	No. of expt.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Monkey.		
Apr. 10	687	Ox 503	—	—	Died; under observation 23 days.
June 26	1168	Ox 1118	—	—	Never showed trypanosomes; still alive after 141 days.
„ 26	1169	„	—	—	Never showed trypanosomes; still alive after 97 days.
			Dog.		
May 21	884	Ox 503	—	—	Died; under observation 66 days.
June 30	1194	Goat 883	—	—	„ „ 42 „
„ 30	1195	„	—	—	Never showed trypanosomes; still alive after 43 days.
„ 30	1196	„	—	—	Died; under observation 26 days.
			Guinea-pig.		
Apr. 10	689	Ox 503	—	—	Never showed trypanosomes; still alive after 59 days.
June 26	1162	Ox 1118	—	—	Never showed trypanosomes; still alive after 36 days.
„ 26	1163	„	—	—	Never showed trypanosomes; still alive after 36 days.
„ 26	1164	„	—	—	Never showed trypanosomes; still alive after 36 days.
			Rat.		
1909. Feb. 9	529	Ox 503	—	—	Experiment stopped; alive after 44 days.
„ 9	530	„	—	—	Experiment stopped; alive after 44 days.
Apr. 10	688	„	—	—	Experiment stopped; alive after 59 days.
„ 12	717	„	—	—	Experiment stopped; alive after 57 days.
June 26	1165	Ox 1118	—	—	Experiment stopped; alive after 57 days.
„ 26	1166	„	—	—	Experiment stopped; alive after 57 days.
„ 26	1167	„	—	—	Experiment stopped; alive after 57 days.
May 21	885	Goat 698	—	—	Experiment stopped; alive after 80 days.
			Mouse.		
Apr. 10	690	Ox 503	—	—	Experiment stopped; alive after 59 days.

* Duration includes the days of incubation; it dates from the day of infection.

CULTIVATION OF T. NANUM.

No attempt was made at Mpumu to cultivate *T. nanum.*

THE CARRIER OF T. NANUM.

Of the two experiments made at Mpumu with *T. nanum* and *Glossina palpalis*, one was unsatisfactory and the other negative. Since the two oxen supposed to be suffering from *T. nanum* disease came from Namukekera, where it is stated there are no tsetse flies, it is probable that *T. nanum*, like *T. pecorum*, is carried by some species of biting fly other than *Glossina.*

CONCLUSIONS.

1. *T. nanum* is indistinguishable from *T. pecorum* either in the living condition or when fixed and stained.
2. It differs from *T. pecorum* in not being pathogenic to the smaller laboratory animals.
3. The carrier of *T. nanum* is probably the same as that of *T. pecorum*, as both diseases occur under the same conditions, but there is no evidence available as to what the carrier is.

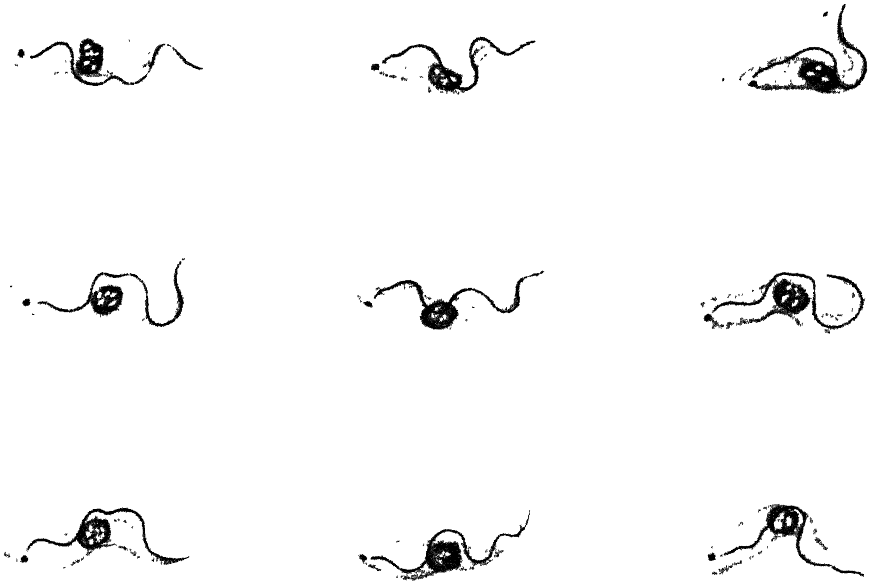
DESCRIPTION OF PLATES.

PLATE 13.

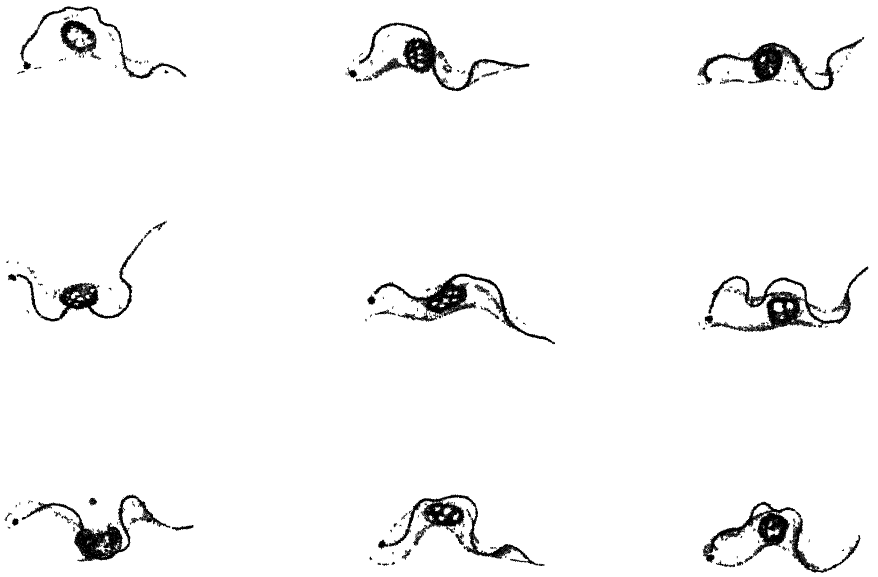
Figures in black and white for the comparison of *Trypanosoma nanum*, Uganda, 1909, and *T. nanum*, Sudan, 1904. × 2000.

PLATE 14.

T. nanum, Uganda, 1909, in the goat. Fixed osmic acid, stained Giesma. × 2000.

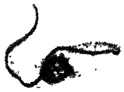


T. Nanum, Uganda, 1909.



T. Nanum. Sudan, 1904.

M.E. Bruce, del.



E. Bruce, del

A Case of Sleeping Sickness studied by Precise Enumerative Methods: Further Observations.

By Major Ronald Ross, F.R.S., and David Thomson, M.B., Ch.B., D.P.H.

(Received October 15,—Read December 8, 1910.)

(1) *Introduction.*

In a previous paper by us, published in the 'Proceedings' of July 21, 1910, B 557, we recorded our observations on this case during two and a-half months, and described particularly the regular periodical rises in the numbers of trypanosomes disclosed in the patient's peripheral blood by methodical daily countings extending over that period. Our technique has been detailed in another paper by us on "Enumerative Studies on Malarial Fever," recently submitted to the Society. We now record our further observations on the case during two more months—until the patient's death. A chart and a table giving daily details of the observations are attached; and accompanying papers by Drs. J. G. Thomson and H. B. Fantham record studies on animals and on the parasites themselves. We must refer also to a recent communication to the Society by Drs. J. W. W. Stephens and Fantham suggesting that the species found in this case may not be identical with *T. gambiense.*

The patient, a strong young Englishman, age 26, weight 154 lbs., was infected in N.E. Rhodesia near the River Luangwa in September, 1909. The trypanosomes were found in his blood in Africa on November 17.

He was admitted into the Royal Southern Hospital on December 4. Daily estimation of the number of trypanosomes per cubic millimetre of his blood was commenced on February 16, and continued till his death on June 29, a period of 134 days. During that period we never failed to find trypanosomes. The history of the case from February 16 is recorded graphically on the folding chart facing p. 198.

The patient had glandular swellings and ill-defined erythematous rashes on his legs at various intervals. On April 20 he had a severe attack of vomiting which continued for four days; in consequence he lost 10 lbs. in weight and was never again so well. He remained in bed onwards till his death. He became progressively more drowsy, with intervals of more or less comparative brightness. His memory and mental powers failed steadily. On May 7 he developed a marked neuritis due to the atoxyl, and every dose seemed to aggravate the neuro-retinitis produced by the previous administration of this drug. His eyes were examined weekly by Dr. Hamilton, oculist to the hospital.

On May 10 both legs became markedly œdematous and remained so till his death. On May 13 he became much worse and developed paralysis of his sphincters. He seemed about to die, but on May 20 a sudden remarkable improvement took place. Soon, however, he again commenced to grow steadily worse.

On June 25 he developed pleurisy and pneumonia. He died on June 29.

Post mortem : performed 22 hours after death :—

Brain: membranes thickened and white. Vessels congested. Cerebrospinal fluid increased.

Left lung: broncho-pneumonia.

Left pleural cavity: contained one pint of yellow pussy fluid.

Spleen: soft and much enlarged; weight, 33 ozs.

Liver: enlarged; weight, 6 lbs.

Blood: no trypanosomes could be found. Leucocytes, 17,000 per cubic millimetre. Polymorph excess.

Smears of bone marrow, spleen, kidney, mesenteric glands, and cerebrospinal fluid, all showed large numbers of small and large mononuclear cells, but no trypanosomes could be detected.

(2) *Continuance of the Periodical Cycle.*

The cycle recorded by us till April 30 in our previous paper continued till his death. From February 22 till June 25 there occurred 19 rises in the numbers of trypanosomes in his peripheral blood. This gives an average of six and a-half days between the heights of each rise.

The shortest period between two successive heights is four days, as recorded between April 18 and 22, and again between June 3 and 7. This exceptionally short interval was due, we think, to artificial means, as we shall explain later under vaccine treatment. The longest period recorded between two successive heights is eight days, while the usual period between the heights seems to be seven days. It will be observed that the rise and fall is sudden, and that the height is not maintained.

While the number of the parasites was increasing rapidly, we observed many forms with double nuclei and blepharoplasts (dividing forms). During the interval between the rises, few of these forms were present. This would seem to indicate that the sudden rise is due to an active multiplication of the parasites.

On April 28 the numbers increased from 200 to 1536 in 24 hours, and on June 19 they increased from 100 to 1500 in 24 hours, suggesting that in our patient they were capable of dividing and sub-dividing three to four times in 24 hours.

The natural fall is even more sudden than the rise. On April 29 the parasites fell from 1536 to 100 per cubic millimetre of blood in 24 hours.

Except on April 5 and 6 only one observation was made daily, so that obviously we may have missed the highest points reached in the various rises. The low levels were, as a rule, maintained for three or four days, forming U-shaped bends as recorded in the chart. The lowest number recorded was 8 per cubic millimetre of blood, while the highest reached 1536 per cubic millimetre of blood.

It is worthy of note that there is a tendency for a high rise to be followed by a low one. This is not without exception, but the three highest rises recorded were followed by the three lowest rises.

(3) *Correlation between the Cycle and the Amount of Fever.*

From the chart one can observe that almost invariably the temperature tends to be higher during the height of the cycle, and that between the rises the temperature keeps lower.

In the table average daily temperatures are recorded. These figures were obtained by taking the average of the four-hourly temperatures observed during the 24 hours of each day. We think that this figure gives a fair representation of the amount of fever for the day, and in this table it may be noted that the average daily temperature rises coincidently with the increase of trypanosomes and falls with their fall.

(4) *Other Clinical Observations and Correlations with the Cycle.*

The pulse rate ranged all through this case from 90 to 120 per minute. During the height of the cycle it tended to increase coincidently with the increased amount of fever. The respiration rate ranged from 20 to 28 per minute, tending also to increase with the rise of the trypanosomes.

With regard to the bowels there is little to note. Their action was easily regulated with small doses of cascara. It was very noticeable that the patient was more drowsy, and tended to have headaches, during the commencement of the cyclical rise, but these symptoms abated at the extreme height and during the subsequent fall of trypanosomes.

The lymphatic glands in the neck, axilla, groin, and popliteal space were more or less always swollen, but this condition was greatly aggravated at intervals, accompanied with marked tenderness, though only sometimes was this coincident with a trypanosome increase.

The urine throughout remained clear, usually slightly acid and without deposits. The specific gravity ranged from 1010 to 1026.

On March 15 he had a diffuse ill-defined erythematous rash over his left leg, occurring just before the height of a trypanosome increase.

(5) *Correlation between the Trypanosome Cycle and the Behaviour of the Leucocytes.*

Previous to April 23 we made several leucocyte counts with the Thomas Zeiss apparatus, but were unable to find anything definite. It was only when we made counts daily at the same hour that we were able to find a definite leucocyte change corresponding to the parasitic cycle.

We counted the leucocytes by means of the thick film as referred to in our paper on malaria, and were astonished at the remarkable variations. We found no such variations by our method in normal persons. Simultaneous examination of thin blood films also showed that a remarkable leucocytic variation did take place, corresponding to each rise of trypanosomes.

Daily differential counts were also made in thin films stained with Giemsa, in which we distinguished only between mononuclears and the so-called polymorphonuclears. In these we never counted less than from 300 to 500 leucocytes to ensure greater accuracy. We thus estimated daily the total leucocytes and the total numbers of polymorphs and mononuclears per cubic millimetre of blood, as shown in the chart and table at end of paper.

From April 23 till May 20 it is clearly seen that coincident with each rise in the number of trypanosomes there is a marked increase in the total leucocytes, and this increase is due more to mononuclears than to polymorphonuclears.

When the trypanosomes begin to increase in number the leucocytes increase also, more especially the mononuclears. When the trypanosomes have reached their height there may be a fall in the number of leucocytes, but this is followed by a still higher rise during the fall of the trypanosomes. The leucocytes would seem to reach their highest numbers on about the third day after the height of the parasitic cycle. They then decrease rapidly for about three days to normal or much lower. When this occurs the trypanosomes again commence to multiply, and the leucocytes again increase also, so that we have both a parasitic and a corresponding leucocytic cycle.

We did not attempt to differentiate between large mononuclears and lymphocytes, but it seemed to us that both took part in the total mononuclear increase. We further observed that during and immediately after the parasitic fall the large mononuclears were filled with vacuoles and a reddish *débris* (stained Giemsa). Many of these vacuolated mononuclear cells were of great size (30 to 40 microns in diameter). We are inclined to think that this vacuolation with reddish stained *débris* may be an indication of the ingestion of trypanosomes, although we have never observed any definite trypanosome structure within them.

F. W. Andrewes, in the Croonian Lectures,* points out that an intravenous injection of bacteria causes a temporary diminution in leucocytes, followed by a marked increase, this fall and rise being chiefly due to the so-called polymorphonuclears, the mononuclears taking little part in the phenomenon. In this disease, as in malaria, it would appear that there is a diminution followed by an increase, chiefly of the mononuclears. This tends to suggest that the polymorphonuclear leucocytes react chiefly to bacterial infections, while the mononuclears would seem to react chiefly to protozoal blood infections (D. T.).

It is interesting to note that on May 20 there was a very marked mononuclear increase, coincident with a fall of trypanosomes, and accompanied by a remarkable improvement in the clinical condition of the patient; and again on June 24 the mononuclear excess changed into a very marked polymorph excess. This was coincident with the onset of pleurisy and pneumonia, from which the patient died.

In connection with the leucocyte cycle, the highest numbers recorded were 50,000 per cubic millimetre of blood, whereas the lowest was 2,800 per cubic millimetre of blood. In the latter part of the chart the leucocytic graph is altered by the injection of leucocytic extract, and by the development of an abscess and pneumonia.

The following quotation from the Sleeping Sickness Bulletin, 1908, No. I, p. 5, is of interest:—"Dr. W. Thomas found that after an injection of atoxyl a change in the parasites became noticeable between the fourth and the fifth hour. They became sluggish and much altered in appearance, and at the same time there was a noticeable increase in the leucocytes. About the seventh hour there was a great diminution in the number of trypanosomes, and a coincident increase in the leucocytes, notably the phagocytes. At the eighteenth hour parasites were absent and could not be found, even after blood centrifugalisation. Instances of phagocytosis were observed on these occasions."

Thomas and Breinl tried the effect of hyperleucocytic agents without good effects, but remark: "It is quite evident that the leucocytes play a rôle in the decrease of parasites."

We think that it is possible that on some occasions, at least, Thomas may ave happened to give atoxyl at the time when the leucocytes were increasing nd the parasites were diminishing, as we have shown to occur naturally.

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* 'Lancet,' July, 1910.

(6) *The Hæmoglobin and Red Cells.*

During the course of the disease from February till the patient's death in June, the hæmoglobin percentage fell more or less steadily from 85 to 70 per cent. We could not detect any variation in the amount of hæmoglobin corresponding to the parasitic cycle.

The number of red cells was deficient (3,800,000 in March; 2,800,000 in June). Their numbers were not estimated frequently.

(7) *The Effects of Treatment by Various Drugs.*

The crucial test of a curative treatment in this disease would naturally be the effect of the treatment on the numbers of parasites, especially in our case, where the numbers were estimated daily.

In testing the value of various therapeutic agents, we have therefore taken the graph of the number of parasites as the indicator of the efficacy of that agent. We would like also to point out that in estimating the effect of treatment by this method, the blood must be examined every day, otherwise the effects recorded might be erroneous. If a drug be given at the height of a parasitic rise, and the blood examined next day or a few days later, the number of parasites would naturally be much less; to conclude from this that the drug has caused this diminution might be quite erroneous. It is obvious that no conclusion should be drawn until the numbers have been estimated daily for several weeks.

We think it may be possible that a drug such as atoxyl may by chance have been given, sometimes, just as the parasites were naturally about to fall, and the rapid diminution attributed to the drug.

It has been stated that atoxyl did not always cause a disappearance of the parasites, but that sometimes instead they even increased in numbers; and further, that it seemed to have a more marked trypanocidal action when the parasites were very numerous.

These statements can be understood in the light of the natural cycle.

Again, if the trypanosomes increase in number immediately after the administration of a drug, one cannot at once conclude that this drug is of no value as a trypanocide.

THE EFFECT OF ATOXYL.

From February 16 till April 5 atoxyl was not given on account of its injurious effect on the patient's eyes. On April 5 we, however, injected a dose of 4 grains at the commencement of a natural rise. The blood was examined every two hours afterwards and showed that the trypanosomes were increasing in numbers. Twenty-four hours later there was a further marked increase. Next day again, as we would have expected to occur naturally, there was a decided diminution in their number.

On May 2 atoxyl was again given, though it was found that every dose aggravated the eye condition. From May 2 till June 20, 32 grains of atoxyl in all were administered at intervals, as noted on the chart. On comparing the graph where no atoxyl was given, and where it was administered, no very appreciable difference in the number of trypanosomes can be detected. We cannot, however, conclude that the atoxyl has no trypanocidal effect, as had it not been given the trypanosomes may possibly have been much more numerous during the latter than in the earlier part of the chart; and further, this particular strain of trypanosome seems to have been very virulent.

Our doses of atoxyl in any case were rather small. It would appear to us, however, that atoxyl cannot be considered a specific in human trypanosomiasis, as quinine is in malaria. In our cases of malaria quinine never failed to diminish, markedly, the number of asexual parasites. Moreover, atoxyl compared with quinine is a dangerous drug.

QUININE AND METHYLENE BLUE TREATMENT.

Quinine 30 grains daily, combined with methylene blue, 12 grains daily, was administered by the mouth from February 21 till March 9, and again at other intervals as noted on the chart. No marked trypanocidal effect can be noticed.

During treatment with these drugs, however, as also with atoxyl, the patient's face, which had before been puffy and œdematous, especially about the eyelids, became more firm and clear cut. The eyelids became again œdematous soon after these drugs were withheld.

It would seem, therefore, that quinine and methylene blue, as well as atoxyl, had some beneficial effect clinically. The following drugs were also given without apparent results:—

(a) *Trypsin and Amylopsin Injections.*—20 min. of each daily.

(b) *Succinamide of Mercury Injections.*—One-fifth grain almost daily.

(c) *Izal Oil.*—8 min. daily, by mouth.

(d) *Trypan Red.*—By the mouth in doses of 0·5 grain, 1 grain, and 1 grain respectively on three successive days. No albuminuria resulted, but the drug was stopped on account of the development of severe vomiting.

(e) *Potassium Iodide.*—30 grains daily, by mouth.

We beg to apologise for complicating the case with so many treatments, but owing to the unsuitability of atoxyl in the patient other treatment was a clinical necessity.

(8) *Effect of Trypanosome "Vaccines."*

On April 19 we commenced to give our patient subcutaneous injections of so-called vaccine. This vaccine was obtained from the blood of a rat inoculated from our patient. The rat was killed when the parasites were extremely numerous (500,000 per cubic millimetre of blood). The blood was then drawn from its heart aseptically, and mixed with an equal volume of normal saline. The red cells were allowed to settle, and the supernatant fluid pipetted off. This latter contained most of the trypanosomes and the number per cubic millimetre was estimated by the thick film method. It was then sterilised by heating to 55° C. for half-an-hour, and by adding trikresol so that it contained 0·2 per cent.

The later vaccines which we commenced to use from May 17 were simply the blood of rats, taken when the trypanosomes were very numerous. They consisted of dead trypanosomes, red cells, leucocytes, and serum. The injection of these vaccines produced no local reaction, even in doses of 100,000,000 trypanosomes; nor were we able to detect any definite temperature reaction.

We are inclined to think that the chief result of these injections of dead trypanosomes was a stimulation of the reproductive powers of the living trypanosomes. This point, however, requires further elucidation. We found that after an injection of our vaccine, the next trypanosome rise usually occurred before it was naturally due.

On April 9, 9,000,000 dead trypanosomes were injected; the next trypanosome rise reached its height on the seventh day. 20,000,000 were again injected on April 13, and the following rise was completed on the sixth day. 40,000,000 were then injected and the next rise was completed on the fifth day.

The patient's trypanosomes, also, which were rising to successively smaller heights, continued to diminish further in number, after two more injections of 10,000,000 dead trypanosomes on April 23 and 25. On April 28, however, an injection of 100,000,000 was given when the trypanosomes were increasing, and that rise was the highest recorded. This treatment was then stopped for some time.

It seems that the effect of these so-called vaccines, if injected immediately after the natural fall of the parasites, is a reproductive stimulation of the parasites, causing the next rise to occur sooner than was natural; and this premature rise tends to be less high than it probably should have been in the natural course of events. If, however, the vaccine be injected during the natural rise of the trypanosomes, it stimulates their reproduction, causing a very high and rapid rise, and the subsequent fall is also very sudden.

We confirmed these surmises later. On May 17 an injection of 30,000,000 dead trypanosomes was given, with the result that the following rise was completed on the sixth day.

Another injection of 50,000,000 on May 30 was followed by a rise at the normal time. A large dose of 100,000,000, injected on June 4, however, scarcely allowed the parasites any time to diminish, so that they completed their next rise on the fifth day, followed by a sudden fall. Thus it would seem that the normal period of seven days between the rises of parasites may be shortened to six, five, and even four days, by an injection of dead trypanosomes.

Doses of vaccine up to 100,000,000 seemed to cause no harm. On the contrary, during the period from the 7th to the 28th of April, when small doses were given, the temperature was more uniform, and more near to normal than it had ever been before or after.

Again, from the 14th to the 19th of May, the patient was extremely ill, almost comatose, with a high temperature, and like to die. On May 17 he had vaccine (30,000,000), followed on May 19 by an injection of 10 c.c. of leucocytic extract. The improvement on May 20 was remarkable, both mentally and physically. Of course, at that time he had atoxyl and nuclein to increase the leucocytes, hence it is almost impossible to tell which was the potent factor. Against atoxyl having caused this good result, we may point out that it had no such effect either before or since.

As already stated, this marked improvement was coincident with a large increase of mononuclear leucocytes. We tried again to repeat this success by injecting vaccine (100,000,000 trypanosomes) on June 4, three days before the height of the parasitic rise, and leucocytic extract at the height of the rise, but the result was not so good (*vide* paper by Ross and J. G. Thomson*).

(9) *The Effect of Subcutaneous Injections of Leucocytic Extract.*

Thinking that the fall in the number of leucocytes might be a factor in the rise of trypanosomes, we determined to keep up the numbers of leucocytes when they were falling. Consequently, we tried an injection of nuclein (25 min.), but this did not seem to cause much increase of leucocytes. Yeast, 15 grains daily (by mouth), was also tried. It seemed to cause some increase, but not marked.

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* *Infra*, pp. 227—234.

At this time Dr. Moore Alexander, pathologist to the hospital, suggested that we should try leucocytic extract. He thought that this extract might contain certain substances which would be deleterious to the trypanosomes, or which would neutralise their endotoxins. He was good enough to prepare it for us by injecting Mellin's food into the pleural cavities of rabbits. The rabbits were then killed, and the accumulation of leucocytes taken from the pleural cavity, and extracted with sterile distilled water.

To our surprise, the effect of an injection of this leucocytic extract was to produce a very great increase of leucocytes in the blood on the day following the injection. This was almost the invariable result. In our opinion, it seems to be a far more powerful promoter of leucocyte increase than yeast or nuclein.

We tried to utilise this discovery in two ways:—

(i) To help the natural leucocyte increase by injecting the extract just at the height of the trypanosome rise. We did this on May 19. Next day, as we have mentioned, the patient showed a remarkable improvement. We were unable, however, to get such a happy result again.

(ii) By injecting the extract a day or two before the rise of parasites, we thought we might prevent that rise by causing an early increase in the leucocytes.

On May 31 10 c.c. of the extract were injected, producing next day an increase of leucocytes up to 20,000 per cubic millimetre of blood, but they diminished next day to 2,800 per cubic millimetre, and the increase of trypanosomes occurred all the same.

Later, just before the patient's death, we tried the effect of 1 c.c. doses of the extract daily. This seemed to keep the leucocyte count high, but unfortunately the results were complicated by the patient developing an abscess at this time, and later by the development of pleuropneumonia.

Further investigation with this substance, and with vaccine, would require to be made before coming to any definite conclusions. Such an investigation we think would lead to further knowledge regarding the natural balance of immunity between the body and the parasites.

It appears to be a law (at least it seems to hold good, we think) with leucocytes and trypanosomes, that the extract of dead animal cells stimulates the corresponding live cells to increase in numbers. This would appear to support the hypothesis put forward by Dr. H. C. Ross, that the extracts of dead tissues promote the proliferation of living cells.*

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* 'Brit. Med. Journ.,' June 11, 1910.

(10) *Conclusions Regarding the Nature and Cause of the Trypanosome Cycle.*

The true explanation of this phenomenon must be of extreme importance, not only as regards the treatment of this disease, but also, we think, with regard to the problems of immunity in general.

Before stating our views we cannot do better than quote the following extract from the Sleeping Sickness Bulletin, 1909, No. 12, p. 485:—

"*Des Causes des Crises Trypanolytiques et des Rechutes qui les suivent.* A. MASSAGLIA. 'Comptes Rendus de l'Académie des Sciences,' Octobre, 1907.

"In some species of animals there are no crises. The trypanosomes increase in a progressive regular manner. In others the trypanosomes, after an increase, suddenly diminish to such an extent sometimes that they cannot be found in the blood. They soon reappear. Massaglia endeavoured to find out the cause of the crisis and the subsequent relapses. . . . *Conclusions:* Trypanolytic crises are due to the formation of anti-bodies in the blood. A few parasites escape destruction, because they become used or habituated to the action of these anti-bodies. These are the parasites which cause the relapses. Since the trypanosomes become more and more used to the anti-bodies the subsequent crises become less marked. . . . Thiroux's suggestion of a balance between anti-bodies and parasites is an interesting one and has some facts in its support. It would serve to explain the cause of real or apparent cures mentioned in Bulletin No. 5, pp. 193 and 195."

Here it is clear that Massaglia has observed and has tried to explain the occurrence of these natural rises and falls of trypanosomes. Our opinions on the subject are as follows:—

(i) *The Cycle is not Due to an Unconditional Cyclical Development of the Parasites as is the Case in Malarial Fever.*—If it were so then one would expect the cycle to be more regular, and one would not expect its time to be altered by vaccine injections. The time between the rises varies not only in the same individual, but in different animals. This would appear to suggest that the cycle is not due to a definite parasitic development as in malaria, but is merely a question of a struggle between the defensive powers of the infected body and the aggressive powers of the trypanosomes.

The more susceptible the animal the shorter is the period between the rises as seen in the case of rats, in which the cycle is almost lost. Massaglia evidently failed to observe the slight remissions in the rat.

The greater the resistance of the infected animal the longer is the cyclical period, as in guinea-pigs and man.

By an "unconditional cycle" we mean one of which the *period* is not affected by the resistance of the host or by therapeutic agents—as, for instance, that of *Plasmodium.* By a "conditional cycle" we mean one which is so affected—as in the present case.

(ii) *The Increase of Parasites is Due to their Sudden and Active Multiplication.*—The presence of numerous dividing forms during the cyclical rise would seem to support this statement.

(iii) *The Multiplication of Trypanosomes is extremely Rapid.*—As before stated they seem capable of dividing in man three to four times in 24 hours.

In rats the rate of multiplication would seem much greater, as many as 10 divisions or generations may occur in 24 hours.

(iv) *The Rate of Multiplication depends on the Suitability of the Infected Blood to the Parasite.*—The blood of rats seems more suitable to the parasite than the blood of man and guinea-pigs.

Also this suitability would appear to vary from time to time in the same animal. In our case there occurred high and low rises in the numbers of trypanosomes. Moreover the three highest rises recorded in the chart were followed by the three lowest rises recorded. This is a very significant fact. It would appear to indicate that a high rise sets up some reactionary condition. This reaction, if great, not only causes the decrease of trypanosomes but extends its influence so far as to reduce the next rise.

(v) *The Fall in Number of Parasites is not Due to the Toxins they Develop.*—In man the numbers may reach only 200 per cubic millimetre of blood and then they diminish, whereas in rats they may reach over 200,000 per cubic millimetre of blood and yet continue to rise, and the toxins in the latter case must be much more abundant than in the former.

It would seem, therefore, that the explanation may be reduced to some of the following causes, all of which may come into play.

(i) *The Increase of Trypanosomes is due to their Active Multiplication,* the rate of multiplication depending on the following conditions:—

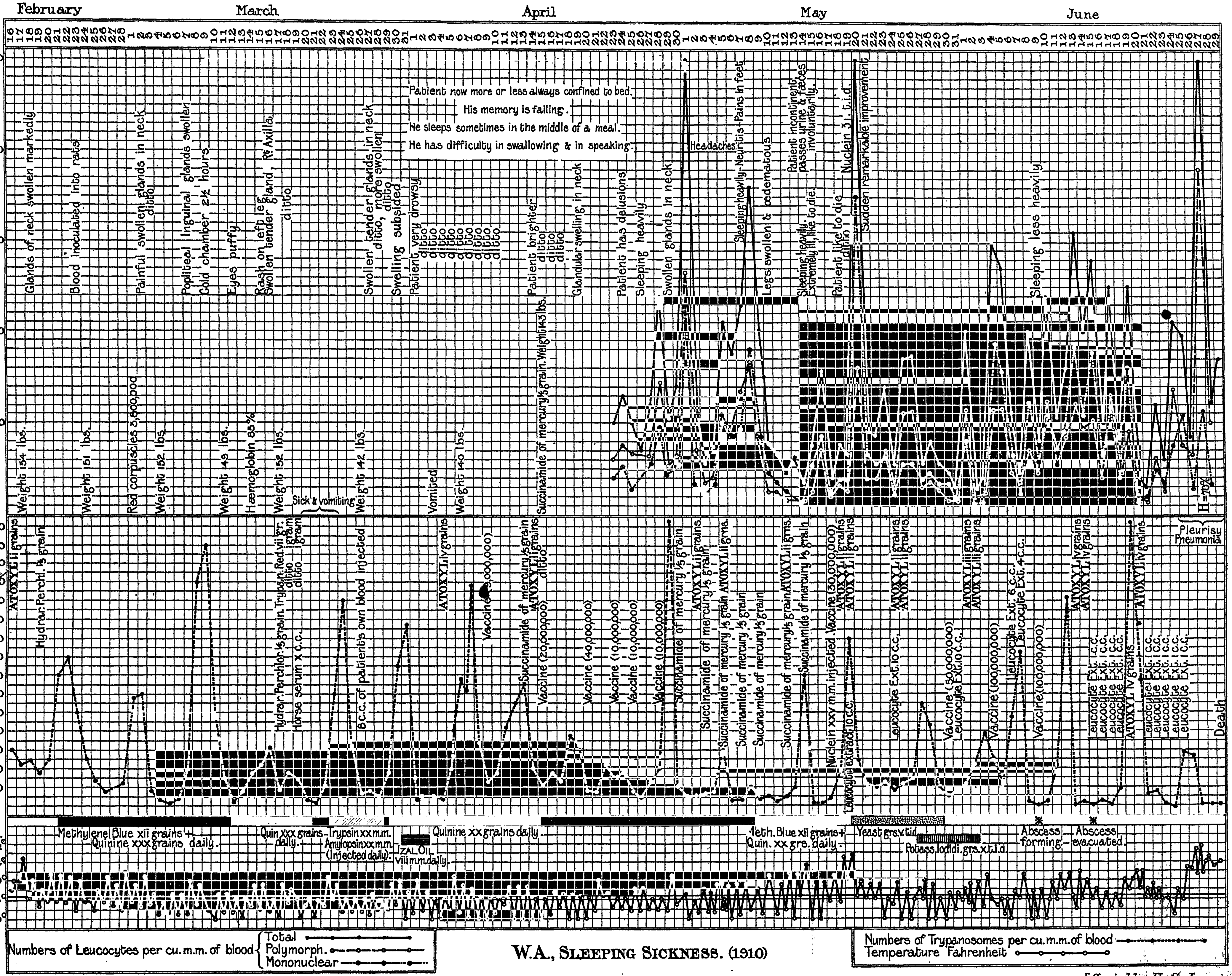
(*a*) The liberation of a reproductive stimulant from the dead trypanosomes of the previous fall.

(*b*) The small number of leucocytes, especially mononuclears.

(*c*) The habituation of the trypanosomes to their anti-bodies.

(*d*) The absence or the diminution of anti-bodies to the trypanosomes.

(ii) *The Decrease of Trypanosomes is Due to their Rapid Death and to a*



W.A., SLEEPING SICKNESS. (1910)

[*Copied by H. S. Leeson.*

Cessation of Multiplication, probably depending on the following conditions :—

(*a*) The presence of anti-bodies in the serum.

(*b*) The large increase of leucocytes, especially mononuclears.

(iii) *The Trypanosomes remaining between the Rises are Resistant Forms*, and tend to become spherical, especially in the internal organs. *Vide* paper by H. B. Fantham.

These resistant spherical forms also occur after atoxyl treatment (Moore and Breinl).

N.B.—The reference to the virulence of this trypanosome and to the cycle in rats and guinea-pigs is taken from the paper on enumerative studies in rats, guinea-pigs, and rabbits by J. G. Thomson and H. B. Fantham.

The virulence of this strain was also noticed by L. E. W. Bevan and Malcolm Macgregor ('Journal of Comparative Pathology and Therapeutics,' June, 1910), who inoculated animals from our patient before he left N.E. Rhodesia.

Table of Daily Details.

W. A.—*Sleeping Sickness.* Infected September, 1909; admitted December, 1909; age 26; male; weight 154 lbs.; died June 29, 1910.

Note.—The parasites are given per c. mm. The temperatures are recorded in the Hæmatothermic Fahrenheit scale, which is the Fahrenheit scale minus 95·0 multiplied by 10. The leucocytes are given in 100's per c. mm.; and the hæmoglobin in Tallquist's scale.

	February.													March.		
Date	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	1.	2.	3.
No. of tryps., per c. mm.	—	166	195	140	296	687	800	500	254	130	70	—	124	570	606	70
Maximum temp., H.F.	60	74	60	40	58	56	54	50	30	40	56	52	56	42	54	36
Average daily temp., H.F.	49	57	46	35	39	44	39	31	29	27	35	28	35	37	35	29
Total leucocytes, in 100's	56	44	39	50	53	—	—	—	62	—	—	—	99	—	119	—
Polymorphs, in 100's	34	26	26	—	—	—	—	—	36	—	—	—	53	—	66	—
Mononuclears, in 100's	22	18	13	—	—	—	—	—	26	—	—	—	46	—	53	—
Mononuclears, per cent.	39	44	36	—	—	—	—	—	42	—	—	—	47	—	45	—
Hb., per cent. (Tallquist)	R.B.C., 3,800,000	—	—	—	—	—	—	—	—	—	—	—	—	—	R.B.C., 3,800,000	—
Treatment	Atoxyl, 2 gr.	—	—	$HgCl_2$, $\frac{1}{5}$ gr.	—	Quinine, 30 gr. Meth. blue, 50 c.c.	*et*	*seq.*	—	—	—	—	—	—	—	—

...	March.															
Date	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.
No. of tryps., per c. mm.	26 ?	12	40	204	1256	1400	472	152	32	76	160	220	320	68	180	132
Maximum temp., H.F.	34	38	36	50	46	34	36	38	36	34	44	46	38	42	40	36
Average daily temp., H.F.	24	24	25	33	33	28	19	27	25	22	32	33	30	29	32	27
Total leucocytes, in 100's	—	—	—	—	116	—	—	—	156	—	—	—	31	47	—	73
Polymorphs, in 100's	—	—	—	—	81	—	—	—	113	—	—	—	17	24	—	43
Mononuclears, in 100's	—	—	—	—	35	—	—	—	43	—	—	—	14	23	—	30
Mononuclears, per cent.	—	—	—	—	—	—	—	—	34	—	—	—	46	49	—	41
Hb., per cent. (Tallquist)	—	—	85	85	80	—	—	—	80	—	85	85	80	75	80	82
Treatment	—	—	—	—	—	—	—	—	Quinine stopped	—	—	—	—	Tryp. red, 5 gr. $HgCl_2$, $\frac{1}{5}$ gr.	Tryp. red, 15 gr.	Tryp. red, 15 gr.

	March.												April.			
Date	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	1.	2.	3.	4.
No. of tryps., per c. mm.	32	24	100	620	1136	322	68	72	32	188	748	1016	144	52	52	40
Maximum temp., H.F.	32	32	40	52	44	38	32	38	28	46	50	42	60	48	32	32
Average daily temp., H.F.	27	21	31	40	37	29	26	24	23	31	37	30	31	35	19	25
Total leucocytes, in 100's	—	62	—	45	73	99	83	56	80	47	41	100	47	81	53	76
Polymorphs, in 100's	—	38	—	29	28	57	34	23	47	25	16	?	21	36	21	37
Mononuclears, in 100's	—	24	—	16	45	42	49	33	33	22	25	?	26	45	32	39
Mononuclears, per cent.	—	40	—	35	62	43	60	59	41	46	62	?	55	56	60	51
Hb., per cent. (Tallquist)	85	85	85	82	85	85	85	—	85	85	85	80	80	80	80	80
Treatment	—	—	—	Trypsin, amylopsin	*et seq.*	—	Vaccine, 1,000,000	—	—	—	Quinine, 20 gr.	*et seqq.* Izal oil, 8 min.	Izal oil, 8 min.	Izal oil, 8 min.	—	—

Table of Daily Details—*continued.*

	April.													
Date	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.
No. of tryps., per c. mm.	212	680	1164	340	116	172	416	556	650	192	96	172	116	392
Maximum temp., H.F.	52	46	40	34	32	30	40	40	40	30	30	38	40	40
Average daily temp., H.F.	37	28	27	23	19	21	33	26	25	21	25	25	24	31
Total leucocytes, in 100's	61	56	89	59	84	53	40	70	62	81	52	?	—	—
Polymorphs, in 100's	33	34	44	35	59	22	19	45	—	41	32	?	—	—
Mononuclears, in 100's	28	22	44	24	25	31	21	25	—	40	20	?	—	—
Mononuclears, per cent.	46	40	50	41	30	58	53	36	—	49	39	47	49	48
Hb., per cent. (Tallquist)	85	85	85	80	80	80	80	80	85	85	85	85	—	85
Treatment	Atoxyl, 4 gr.	—	—	—	Vaccine, 9,000,000	—	—	—	Succ. Hg, $\frac{1}{5}$ gr.	Atoxyl, 2 gr.	Atoxyl, 2 gr. Vaccine, 2,000,000	—	—	Succ. Hg, $\frac{1}{5}$ gr.

	April.											
Date	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.
No. of tryps., per c. mm.	336	90	72	244	156	164	88	48	140	200	1536	120
Maximum temp., H.F.	36	32	52	44	34	32	38	38	32	32	52	42
Average daily temp., H F.	23	22	31	34	27	24	27	27	24	25	32	30
Total leucocytes, in 100's	100	—	—	—	100	130	100	72	120	240	108	140
Polymorphs, in 100's	52	—	—	—	60	78	70	—	64	144	52	66
Mononuclears, in 100's	48	—	—	—	40	52	30	—	56	96	56	74
Mononuclears, per cent.	48	36	31	52	40	40	30	—	46	40	52	53
Hb., per cent. (Tallquist)	85	—	80	85	80	80	80	80	85	80	85	—
Treatment	—	Quinine stopped. Vaccine, 40,000,000	—	—	Vaccine, 10,000,000	—	Vaccine, 10,000,000	—	—	Vaccine, 10,000,000	—	Succ. Hg, $\frac{1}{5}$ gr.

	May.										
Date	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
No. of tryps., per c. mm.	64	64	68	76	292	8	40	108	56	64	8
Maximum temp., H.F.	38	34	40	48	44	46	46	34	54	44	46
Average daily temp., H.F. ...	24	28	30	34	32	24	27	26	35	25	31
Total leucocytes, in 100's	500	110	53	73	210	174	230	350	150	75	62
Polymorphs, in 100's............	265	70	33	40	101	87	138	168	63	25	25
Mononuclears, in 100's	235	40	20	33	109	87	92	182	87	50	37
Mononuclears, per cent..........	47	36	38	45	52	50	40	52	58	66	59
Hb., per cent. (Tallquist)......	85	80	80	80	80	80	80	80	80	80	80
Treatment	—	Atoxyl, 2 gr.	Succ. Hg, $\frac{1}{5}$ gr.	—	Succ. Hg, $\frac{1}{5}$ gr. Atoxyl, 2 gr.	—	Succ. Hg, $\frac{1}{5}$ gr.	—	Succ. Hg, $\frac{1}{5}$ gr. Quin. et Meth. blue.	—	—

	May.									
Date	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.
No. of tryps., per c. mm.	76	100	700	8	4	52	176	900	208	104
Maximum temp., H.F.	56	56	68	48	48	56	70	78	64	42
Average daily temp., H.F. ...	38	36	48	30	34	41	47	64	45	35
Total leucocytes, in 100's	45	63	37	75	157	36	72	68	500	100
Polymorphs, in 100's............	16	—	33	21	71	18	33	29	160	38
Mononuclears, in 100's	29	—	33	54	86	18	39	39	340	62
Mononuclears, per cent.	64	—	50	72	55	49	54	57	68	62
Hb., per cent. (Tallquist)......	80	75	80	80	80	80	80	80	80	85
Treatment	Succ. Hg, $\frac{1}{5}$ gr. Atoxyl, 2 gr.	—	Succ. Hg, $\frac{1}{5}$ gr.	—	—	Vaccine, 30,000,000 Nuclein, 25 min.	Atoxyl, 2 gr.	Atoxyl, 2 gr. Leuc. ext., 10 c.c.	—	—

Table of Daily Details—*continued.*

	May.										June.
Date	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	1.
No. of tryps. per c. mm.	104	112	68	120	150	552	416	100	48	72	128
Maximum temp., H.F.	42	42	44	36	30	50	42	34	36	36	44
Average daily temp., H.F.	36	28	31	23	24	38	27	27	25	27	34
Total leucocytes, in 100's	96	140	53	170	175	54	80	57	43	30	200
Polymorphs, in 100's	39	71	30	114	117	21	34	26	12	13	116
Mononuclears, in 100's	57	69	23	56	58	33	46	31	31	17	84
Mononuclears, per cent.	59	49	44	33	33	62	58	55	72	56	42
Hb., per cent. (Tallquist)	85	—	85	85	85	85	85	85	80	80	80
Treatment	—	—	Atoxyl, 2 gr. Leuc. ext., 10 c.c.	Atoxyl, 2 gr.	—	—	—	—	Vaccine, 50,000,000	Leuc. ext., 10 c.c.	Atoxyl, 3 gr.

	June.										
Date	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
No. of tryps. per c. mm.	240	352	248	112	468	820	20	12	24	208	1164
Maximum temp., H.F.	44	56	54	30	38	56	40	40	46	56	58
Average daily temp., H.F.	34	33	36	21	24	39	27	26	30	41	48
Total leucocytes, in 100's	28	40	300	270	110	45	200	80	240	108	160
Polymorphs, in 100's	14	23	114	119	45	27	72	33	130	49	74
Mononuclears, in 100's	14	17	186	151	65	18	128	47	110	59	86
Mononuclears, per cent.	50	43	62	56	59	40	64	59	46	55	54
Hb., per cent. (Tallquist)	80	80	80	80	80	80	80	80	80	80	80
Treatment	Atoxyl, 3 gr.	—	Vaccine, 100,000,000	—	Leuc. ext., 6 c.c.	Leuc. ext., 4 c.c.	—	Vaccine, 100,000,000	—	—	—

	June.							
Date	13.	14.	15.	16.	17.	18.	19.	20.
No. of tryps. per c. mm.	16	32	8	32	16	100	1452	680
Maximum temp., H.F.	60	58	50	38	38	50	50	60
Average daily temp., H.F.	48	41	40	27	28	37	44	45
Total leucocytes, in 100's	310	200	280	100	250	81	250	37
Polymorphs, in 100's	124	92	101	40	125	39	90	29
Mononuclears, in 100's	186	108	179	60	125	42	160	16
Mononuclears, per cent.	60	54	64	60	50	52	64	43
Hb., per cent. (Tallquist)	80	80	—	—	80	—	80	80
Treatment	Atoxyl, 4 gr.	Atoxyl, 4 gr.	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	Atoxyl, 4 gr.	Atoxyl, 4 gr.

	June.								
Date	21.	22.	23.	24.	25.	26.	27.	28.	29.
No. of tryps. per c. mm.	56	72	40	24	300	260	40	40	30
Maximum temp., H.F.	42	42	34	40	66	88	78	74	70
Average daily temp., H.F.	35	34	30	27	42	75	70	69	70
Total leucocytes, in 100's	32	120	60	210	195	84	500	126	—
Polymorphs, in 100's	13	61	28	147	78	66	380	105	—
Mononuclears, in 100's	19	59	32	63	117	18	120	21	—
Mononuclears, per cent.	60	49	54	35	60	21	24	17	—
Hb., per cent. (Tallquist)	80	70	70	70	75	70	70	70	—
Treatment	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	—	—	—	—

Enumerative Studies on Trypanosoma gambiense *and* Trypanosoma rhodesiense *in Rats, Guinea-pigs, and Rabbits; Periodic Variations disclosed. (Preliminary Note.)*

By H. B. Fantham, D.Sc., B.A., and J. G. Thomson, M.A., M.B., Ch.B.

(Communicated by Major R. Ross, C.B., F.R.S. Received October 15,—Read December 8, 1910.)

The investigations were undertaken at the suggestion of Major R. Ross, under funds allotted by the Tropical Diseases Research Committee in the case of one of us (H. B. F.) and under the Sir Edwin Durning-Lawrence Fund in the case of the other (J. G. T.).

The researches were necessary in order to determine whether a regular periodic increase in the trypanosomes in the peripheral circulation of sub-inoculated animals occurred, like that found by R. Ross and D. Thomson (1910)* in a case of Rhodesian Sleeping Sickness, and whether this numerical cyclical development had a definite significance in the life-history of the parasite, which it would be necessary to consider in the case of treatment by drugs or other methods. Our work was done in the Liverpool School of Tropical Medicine.

The trypanosome from the patient suffering from Sleeping Sickness contracted in Rhodesia was found by Stephens and Fantham (1910)† to exhibit a marked morphological peculiarity in the presence of a posterior nucleus, and for this parasite the name *T. rhodesiense* was suggested by them. We have also used an old laboratory strain of *T. gambiense*.

In all cases a definite number of trypanosomes were inoculated. The inoculation was made subcutaneously in rats, intra-peritoneally in guinea-pigs and rabbits. The peripheral blood of these animals was then examined daily at the same hour, that is to say at regular intervals of 24 hours.

The technique employed was that elaborated by R. Ross and D. Thomson in their study of the patient W. A., namely, that a known quantity of blood (1 cubic millimetre divided into quarters) was taken and the parasites therein very carefully counted. In counting the thick films, an Ehrlich eyepiece was used, after the films had been dehæmoglobinised, fixed with absolute alcohol, and stained by the Romanowsky method. When the parasites were very numerous, it was necessary to spread the quarter cubic millimetre of blood over a larger square area than usual, and to use a small diaphragm in the

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* 'Roy. Soc. Proc.,' B, vol. 82, pp. 411—415.

† 'Roy. Soc. Proc.,' B, vol. 83, pp. 28—33.

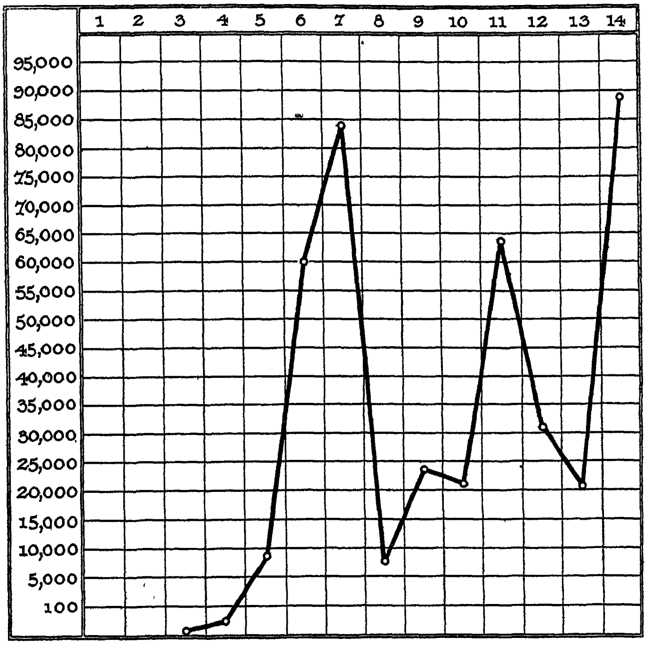

Chart 1.—Graph showing Daily Counts of the Parasites, per c. mm., in the Peripheral Blood of a Rat inoculated with *T. rhodesiense*. Periodic Variation.

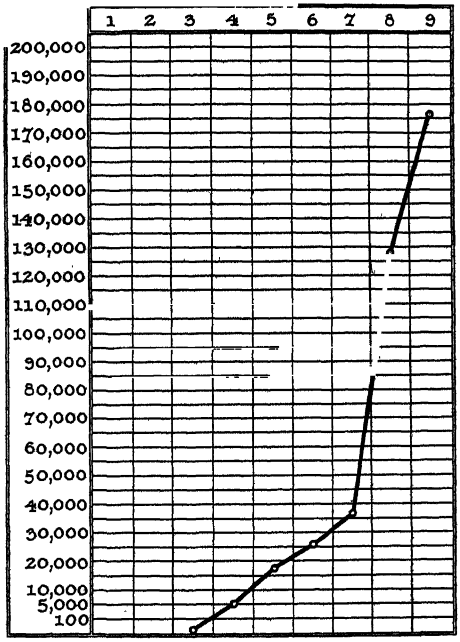

Chart 2.—Graph of Daily Counts of the Parasites in the Blood of a Rat inoculated with *T. rhodesiense*. Continuous Rise.

epiece. The parasites were then counted in three or four bands across e film, which had been previously examined in order to ascertain an even istribution of the parasites therein.

Rats.—In the case of both strains it was found that the inoculated rats nded to fall into one of two main categories, exhibiting either (1) a periodic icrease and decrease in the number of parasites in the peripheral blood (see hart 1), or (2) a more or less continuous rise in the numbers of parasites ntil the animal died (see Chart 2).

Daily counts were made of 17 rats exhibiting periodic variation, and of ive rats exhibiting continuous increase in the case of *T. rhodesiense*. Further, imilar counts were made of seven rats exhibiting periodic variation after noculation with *T. gambiense* (see Chart 3) and of four showing continuous ncrease in the case of *T. gambiense.*

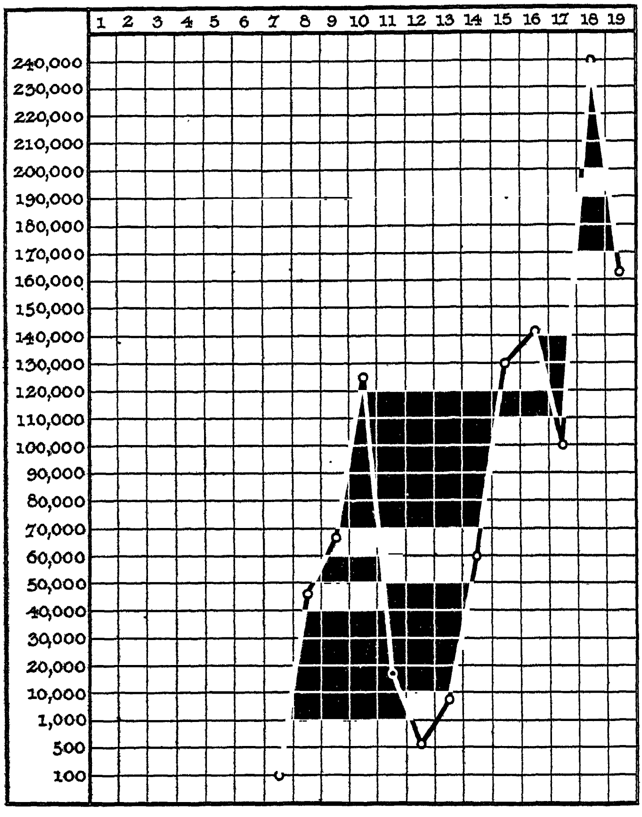

Chart 3.—Graph showing Daily Counts of the Parasites, per c. mm., in the Peripheral Blood of a Rat inoculated with *T. gambiense*. Periodic Variation.

The average incubation period in rats in the case of *T. rhodesiense* was 2·9 days, whereas in the case of *T. gambiense* it was 4·4 days. The average number of parasites inoculated in the case of each strain was approximately the same.

The average life of rats inoculated with *T. rhodesiense* was 11·3 days, whereas the life of rats infected with *T. gambiense* was 13·8 days. (The average weight of the rats inoculated with *T. rhodesiense* was 138·8 grammes, of those infected with *T. gambiense*, 137 grammes.)

The number of divisions of parasites occurring in the first 24 hours in both strains averaged 6, and varied in each case from 2 to 10 divisions, being most frequently about 8.

There is a shorter period between the crests of the waves in the graphs denoting the number of trypanosomes in the Rhodesian strain (about three to four days) than in the laboratory strain of *T. gambiense* (namely, four to six days). The Rhodesian strain, then, was the more virulent.

Guinea-pigs.—Daily counts were made of five guinea-pigs inoculated with *T. rhodesiense* (see Chart 4), and of five guinea-pigs inoculated with *T. gambiense* (see Chart 5). The duration of life (averaging 59 days) of those inoculated with the Rhodesian strain was shorter than in those inoculated with *T. gambiense* (averaging 111 days). In guinea-pigs the disease tended to run a chronic course. The periods between the crests of the waves in both strains in guinea-pigs were longer than in rats, namely, five to eight days, and the initial rise was slower.

Rabbits.—Two rabbits were inoculated with *T. rhodesiense.* They lived 29 and 24 days respectively. Periodic variation occurred in the numbers of the parasites, especially in one case. These animals exhibited an affection of the eyes and skin, there being a white purulent discharge from the eyes, and the skin at the roots of the ears became encrusted with scabs.

The periodicity in the numbers of the parasites in the peripheral circulation of infected animals is to be explained by (*a*) variations in resistance on the part of the host, probably due to the formation of anti-bodies; accompanied by (*b*) the formation on the part of the trypanosomes of rounded, latent, non-flagellate (relatively resistant) forms in the internal organs of the host during the fall in numbers of the parasites in the peripheral blood.

There is, therefore, a life-cycle of trypanosomes in the vertebrate host, in addition to stages of the parasite in the invertebrate carrier (e.g. *Glossina*).

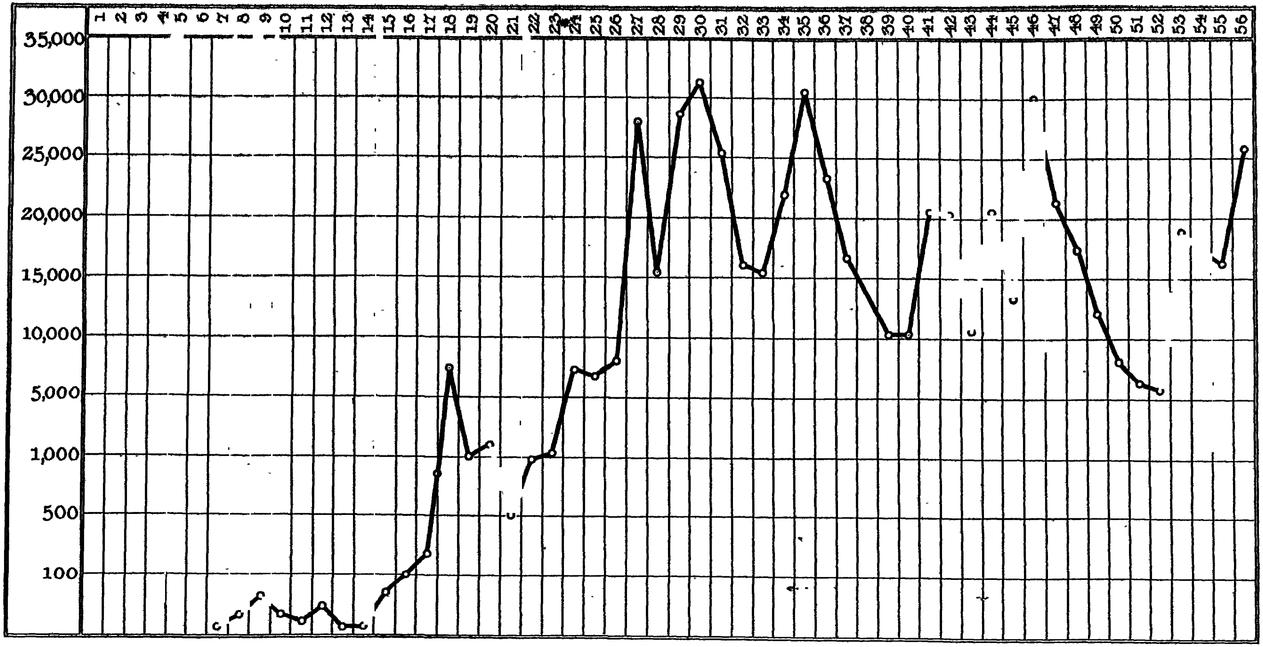

CHART 4.—Graph of Daily Counts of the Parasites in the Blood of a Guinea-pig inoculated with *T. rhodesiense*. Periodic Variation.

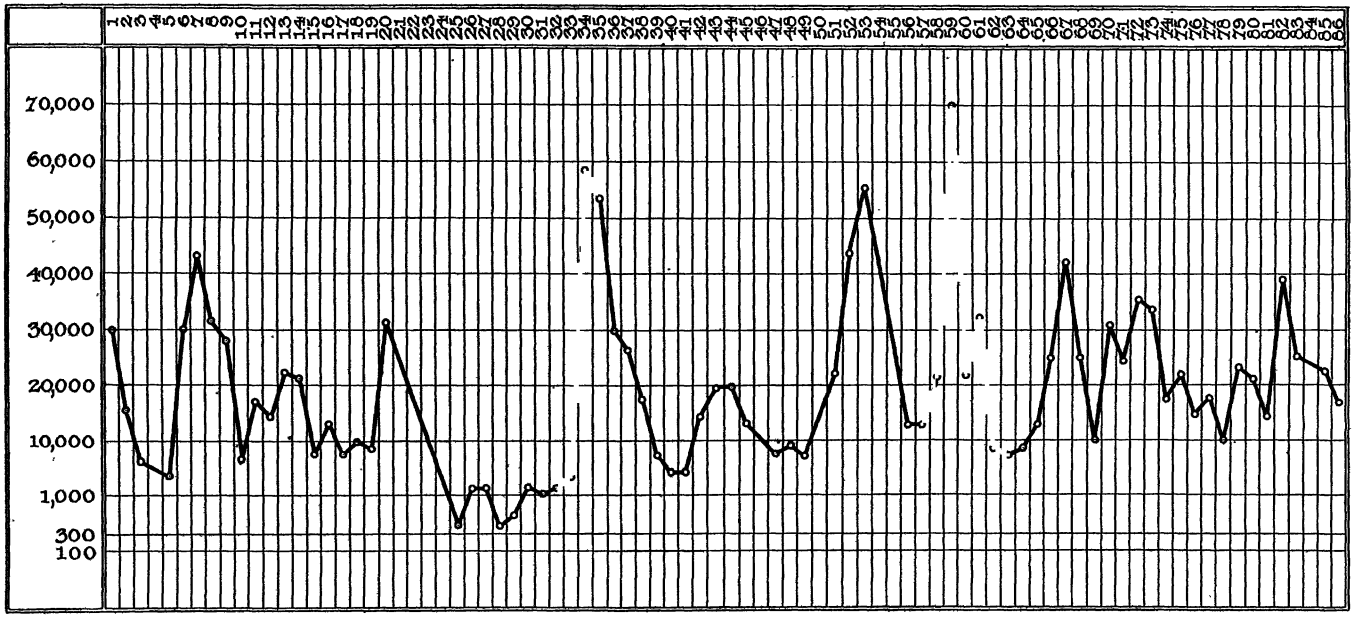

CHART 5.—Graph of Daily Counts of the Parasites in the Blood of a Guinea-pig inoculated with *T. gambiense*. Periodic Variation.

The Life-History of Trypanosoma gambiense *and* Trypanosoma rhodesiense *as seen in Rats and Guinea-pigs.*

By H. B. Fantham, D.Sc. Lond., B.A. Cantab., A.R.C.S.

(Communicated by Prof. Major R. Ross, C.B., F.R.S. Received October 15,—Read December 8, 1910.)

[Plate 15.]

Introduction.

The researches recorded in this paper were undertaken at the suggestion of Major Ross, who wished me to investigate the parasitological aspect of the numerical cyclical development discovered by him and Dr. D. Thomson (1910) in the trypanosome occurring in a patient suffering from Sleeping Sickness contracted in Rhodesia, particularly as regards the possible connection of the latent bodies of Salvin-Moore and Breinl (1907) with that cycle. The investigations have been conducted in the Liverpool School of Tropical Medicine, under a grant from the Tropical Diseases Research Fund.

A complete and generally accepted life cycle of *Trypanosoma gambiense* has yet to be written. The following paper is offered as a contribution to the solution of this difficult problem, and deals with that portion of the life history of the parasite which takes place in a Vertebrate host.

The sub-inoculations in animals—rats and guinea-pigs—recorded herein were made from a patient suffering from Rhodesian Sleeping Sickness in Prof. Ross's clinic in the Royal Southern Hospital, Liverpool. The trypanosome from this source showed a marked morphological feature in the possession of a posterior nucleus in some forms, and for this parasite the name *T. rhodesiense* has been suggested by Stephens and Fantham (1910). A laboratory strain of *T. gambiense* was also used in these investigations for comparison.

Special attention has been paid to the observation of the *living* parasite, as well as to stained preparations.

Methods.

Fresh preparations of *T. gambiense* and *T. rhodesiense* were made from blood taken aseptically from animals, namely, tame rats and guinea-pigs. The blood was kept in sealed cover-slip preparations, at or below blood heat (25° to 37° C.), and also at laboratory temperature. The blood was sometimes diluted with a little physiological salt solution, or with a little isotonic sodium citrate solution. Methylene blue was sometimes used for *intra vitam* staining.

Wet preparations of the parasite were made on cover slips after fixation with osmic acid vapour or corrosive acetic alcohol. The chief stains used were iron hæmatoxylin and those of Giemsa and Romanowsky.

Dry smears were employed for rough work.

In animals killed at certain stages of infection, the various internal organs were carefully examined both in fresh preparations and stained smears.

The enumerative methods used were those recently employed by R. Ross and D. Thomson and used by various workers in Liverpool, being an elaboration of R. Ross's thick-film method made with measured quantities of blood (1 cubic millimetre divided into quarters). The films were dehæmoglobinised, fixed in absolute alcohol, and stained by the Romanowsky method.

Resumé of Previous Work on Latent Bodies of Trypanosomes.

The various flagellate forms of *Trypanosoma gambiense* have been so often described by many competent workers that it is needless to discuss them further in detail. Suffice it to say that long, thin trypanosomes may occur, especially at the beginning of infection in rats and guinea-pigs, and shorter, stout or stumpy forms later. Trypanosomes intermediate in character also are found.

The so-called rounded, latent or encysted forms, which are non-flagellate, must be discussed in greater detail. Marked attention to these non-flagellate forms of trypanosome was first drawn by Moore and Breinl (1907–8) in mammalian trypanosomes, though Dutton saw rounded forms of an Amphibian trypanosome on the Congo in 1903–5. Moore and Breinl (1907), working on stained material, stated that latent forms occurred in the internal organs especially during the periods when the flagellates were decreasing or absent from the peripheral blood of the host. These authors give a curve showing the variations in the numbers of *T. gambiense* in infected rats; they do not, however, give any numerical data in support of the graph. A few other workers have mentioned rounded bodies in connection with trypanosomes, among whom Hindle (1909) may be noted. The significance of the latent bodies is still a disputed point, and it was recently stated in a review that more evidence was required to show that they "constitute part of a life-cycle in the Vertebrate host."*

In the present paper the general statements of Moore and Breinl, regarding forms of *T. gambiense* in the internal organs of rats, are shown to be accurate, and many details, as seen in the living parasite, as well as numerical data from daily counts of the trypanosomes, are supplied in proof of the significance of the latent bodies. Animals inoculated with *T. rhodesiense* (Stephens and Fantham) have also been carefully investigated.

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* Sleeping Sickness Bureau, Bulletin No. 15 (March, 1910), p. 102.

The Relation between Latent Bodies and the Number of Trypanosomes in the Peripheral Blood, with Notes on the Parasites found in the Internal Organs of the Hosts.

Following on the work of R. Ross and D. Thomson (1910) on the enumeration of the parasites in the peripheral blood of a patient suffering from Rhodesian Sleeping Sickness, the periodic increase in the number of trypanosomes in the peripheral blood of rats and guinea-pigs inoculated with *T. rhodesiense* or with *T. gambiense* has recently been found by Fantham and J. G. Thomson.

Rats similarly inoculated and exhibiting such periodic increase and decrease were killed at various points in the cycle, as set forth in the following tables:—

A. Rat 1.—*T. gambiense,* Laboratory Strain, in Piebald ♂ Rat, weight 150 grammes, inoculated with 50,000 trypanosomes.

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Parasites, per c. mm.	—	—	—	—	—	—	4	1088	4392	868	1656	2560	9288	6304	436, killed
Temp.*		55	46	70		62	57	54	60	68	44	46	57	54	61

* The temperature is expressed according to the following convention, as in Major Ross's recent papers on "Malaria" and "Trypanosomiasis":—Temp. = (F−95) × 10, where F is the temperature in degrees Fahrenheit, recorded with a clinical thermometer.

Heart blood of Rat 1 examined fresh, immediately after killing: a few living trypanosomes seen and some (fewer) rounded forms.

Spleen: very large; three to seven rounded forms seen in every field of the microscope (2 mm. objective and 2 ocular); no flagellates seen in fresh preparation.

Liver and portal blood: living flagellates and rounded bodies seen in about equal numbers (one of each per field).

Lungs: a few living flagellates seen, four times as many rounding and rounded bodies seen [100 parasites counted in this and subsequent fresh preparations, when possible].

Bone marrow: many rounded bodies.

Rat 2.—*T. gambiense*, Laboratory Strain, in White ♀ Rat, weight 128 grammes, inoculated with 200,000 trypanosomes.

Day	1	2	3	4	5	6	7	8	9	10	11	12
Parasites, per c. mm.	—	—	—	—	4	684	3640	7392	10,000	6320	1840	2200, killed
Temp.	38		40	45	50	54	60	55	58	44	46	48

The internal organs of Rat 2 were in much the same condition as in Rat 1; further details are superfluous.

B. Rat 3.—*T. rhodesiense* in Piebald Rat, ♀, weight 108 grammes, inoculated with 350,000 trypanosomes.

Day	1	2	3	4	5	6	7	8	9	10
Parasites, per c. mm.	—	—	172	18,160	42,120	31,040	21,600	12,844	130,000	61,440, killed
Temp.	22	20	20	40	56	36	20	21	15	14

Heart blood, fresh, of Rat 3: free trypanosomes seen.

Spleen: many rounded bodies; no free trypanosomes seen.

Liver: a few free trypanosomes seen.

Lungs: about equal numbers of flagellates and rounded bodies.

Bone marrow: rounded bodies.

Rat 4.—*T. rhodesiense* in White Rat, ♂, weight 120 grammes, inoculated with 600,000 trypanosomes.

Day	1	2	3	4	5	6	7	8	9	10	11
Parasites, per c. mm....	—	—	4	896	2880	31,360	51,200	64,000	22,820	12,500	6144, killed
Temp.		20	25	38	50	50	48	47	13	20	31

Heart and liver of Rat 4: many large trypanosomes seen in fresh preparations, very few rounded bodies.

Lungs: a few free flagellates, three times as many rounded forms.

Spleen: a few small flagellate trypanosomes (3 per cent.); many rounded forms.

Kidney blood: flagellate trypanosomes.

Bone marrow: rounded bodies.

Rat 5.—*T. rhodesiense* in White Rat, ♀, weight 247 grammes, inoculated with 120,000 trypanosomes.

Day	1	2	3	4	5	6	7	8	9	10
Parasites, per c. mm.	—	—	—	—	4	12	20	296	624	3744, killed
Temp.			52	38	34	96	86	86	20	66

Flagellate trypanosomes seen in all the internal organs of Rat 5. A few latent bodies seen in the lungs, spleen, and bone marrow.

Rat 6.—*T. rhodesiense* in White Rat, ♂, weight 150 grammes, inoculated with 500,000 trypanosomes.

Day	1	2	3	4	5	6	7	8	9
Parasites, per c. mm.	—	—	—	—	72	2056	1968	6420	592, killed
Temp.	38	36	51	56	54	70	70	62	54

Heart blood, fresh, of Rat 6: flagellate parasites seen, only three latent bodies found in a fresh preparation.

Liver: flagellates 96, and latent bodies 4; in one hour, however, the number of latent bodies in the fresh preparation had increased 16 times (namely to 64), and the flagellates were correspondingly fewer.

Lungs: about equal numbers of trypanosomes and latent bodies.

Spleen: many latent bodies, no free flagellates seen.

Bone marrow: a few latent bodies, no flagellates seen.

Rat 7.—*T. rhodesiense* in Piebald Rat, ♀, weight 170 grammes, inoculated with 1,000,000 trypanosomes. Weight 182 grammes at death, but pregnant.

Day...............	1	2	3	4	5	6
Parasites, per c. mm. ...	—	—	8	5852	27,916	16,468, killed
Temp.	38		62	55	67	66

Heart blood of Rat 7: many flagellates, few rounded bodies.
Liver: equal numbers of flagellates and rounded bodies.
Lungs: three times as many latent bodies as flagellates.
Bone marrow: large rounded bodies.
Spleen: no flagellates seen, but many rounded bodies
Splenic vein blood: twice as many flagellates as rounded bodies.
Mesenteric gland fluid: many flagellates.
Lymph: rounded bodies and Crithidia-like forms.
Placental blood: living flagellates, few rounded bodies.
13 embryos: serous fluid contained a very few trypanosomes.
Embryonic liver: no flagellate trypanosomes seen.

From counts of the number of trypanosomes in the peripheral blood daily, and from examination of carefully prepared smears of organs, it is found that latent bodies are most numerous when the flagellate parasites are few. If inoculated animals be killed at these periods, very few flagellate trypanosomes are found in the spleen and bone marrow (see preceding tables), but many latent bodies are present in those organs, while rounding forms are seen especially in the lungs.

In the peripheral blood, on the upward slope of the curve representing the numbers of the parasites from day to day, the parasites increase in numbers by longitudinal division to a maximum. At or about this period, the formation of rounded or latent bodies begins, and takes place especially in the internal organs.

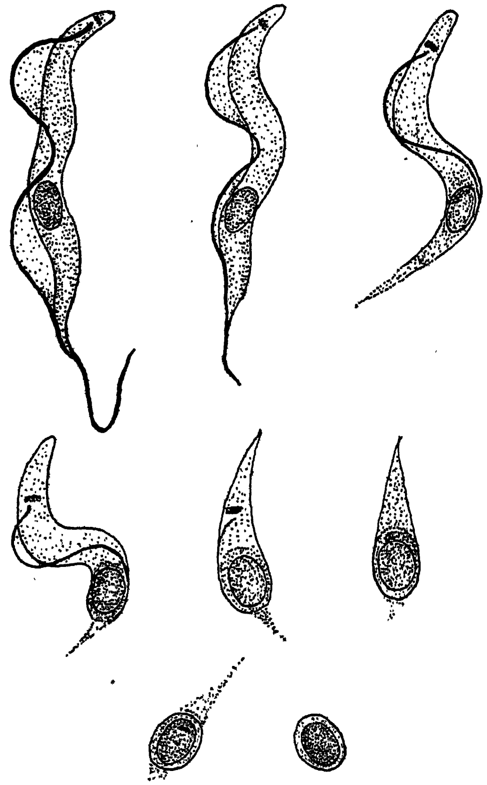
If rounded (latent) bodies, derived from the internal organs of an infected rat, be placed in warm fresh blood drawn from a normal, uninfected rat, then growth of some of the rounded bodies towards the flagellate trypaniform stage can be seen under the microscope, as is detailed in a subsequent section of this paper.

Five guinea-pigs (three inoculated with *T. rhodesiense* and two with *T. gambiense*), dying in various stages of trypanosomiasis, were carefully examined, and fresh preparations and smears of their internal organs were made. Rounding and rounded forms of trypanosomes were seen, just as in infected rats.

Needless to say, a very careful examination of the internal organs of normal (uninfected) rats and guinea-pigs was made for the purposes of comparison and control.

The Formation of Latent Bodies from Flagellate Trypanosomes.

This is diagrammatically represented in text-fig. 1, in which the formation of a rounded body from a living trypanosome was observed under the microscope in a drop of blood and lymph from the tail of an infected rat. The time taken for the formation of the rounded body was 30 minutes.



TEXT-FIG. 1 represents diagrammatically the formation of a latent (non-flagellate) body of *Trypanosoma gambiense* in infected rat's blood, as seen under the microscope on a warm stage, during a period of 30 minutes.

The intervals were 3, 8, 15, 20, 23, 25, and 30 minutes respectively from the commencement of change of form of the parasite. Intranuclear karyosome not represented.

In this process the anterior or flagellar end disintegrates and is cast off, while at the other end the blepharoplast (kinetonucleus) gradually migrates nearer the nucleus, and then the non-flagellar or posterior end of the original trypanosome is cast off, together with the remains of the flagellum. The rounded body, consisting of chromatin with a thin layer of cytoplasm, has then surrounded itself with a definite, very thin capsule ("cyst"). This process, as seen under the microscope, is either the natural mode of formation of non-flagellate bodies in the internal organs or closely approximates thereto, as intermediate stages exactly similar in the formation of these bodies are seen in stained preparations of the heart, lungs, and spleen (Plate 15, figs. 1–5).

However, in the peripheral blood there occur rounded, oval or somewhat pyriform parasites (figs. 17–20), each with a single anterior flagellum. Such forms may, for convenience, be called rounding herpetomonad forms, as Herpetomonas passes through similar stages in assuming a rounded non-flagellate form. The rounded stages of Herpetomonas, thus formed, have been aptly termed post-flagellate stages by Captain Patton and by Dr. Annie Porter in their recent interesting researches on flagellates (Crithidia and Herpetomonas). The rounded, encapsuled stages of trypanosomes are post-flagellate stages. That these rounded, post-flagellate forms are encapsuled in a thin membranous structure is shown by the fact that they resist maceration in water much longer than the trypaniform flagellates.

The formation of post-flagellate bodies (figs. 2, 3, 5) is well seen in the lungs, whence they find their way in the blood stream to the spleen and bone marrow.

The Structure of Latent Bodies.

The post-flagellate (latent) stages of *T. gambiense* and *T. rhodesiense,* already mentioned, have a relatively simple structure. The strictly latent or non-flagellate forms are usually oval in outline, and small, about 2 μ to 4 μ in diameter (figs. 2–16). Less frequently are they quite rounded or spherical (fig. 14), while sometimes they are pyriform (figs. 25, 27, 29, 30). Internally there is a nucleus, which may show a karyosome, and beside the nucleus there is a blepharoplast or kinetonucleus (fig. 6). In some latent bodies. especially the smallest ones, the kinetonucleus may not be visible separately (figs. 11, 13–15), as it may be lying over the nucleus, or actually affixed thereto (figs. 2, 10). The juxtaposition of the nuclear bodies has been actually observed in some Romanowsky-stained specimens, after careful wet fixation (figs. 2, 10). The relative positions of the nucleus and blepharoplast in rounding or rounded bodies may vary considerably. A small quantity of cytoplasm occurs in the latent bodies.

In the formation of rounded, latent bodies, as seen *in vitro*, a portion of the body of the flagellate, after passage of the blepharoplast towards the nucleus, is thrown off, and the flagellum is discarded (text-fig. 1). Examination of preparations of the internal organs (lungs, heart, spleen) of the host shows that a similar method of formation of the post-flagellate stages of the parasite usually occurs in the internal organs (figs. 1–5). However, in the peripheral blood, after careful searching of a sufficient quantity of blood ($\frac{1}{4}$ cubic millimetre), a few rounding or rounded parasites can generally be seen (figs. 12, 17–20). Also, in the internal organs, stages of the parasite intermediate between flagellate and non-flagellate forms may be seen (fig. 33).

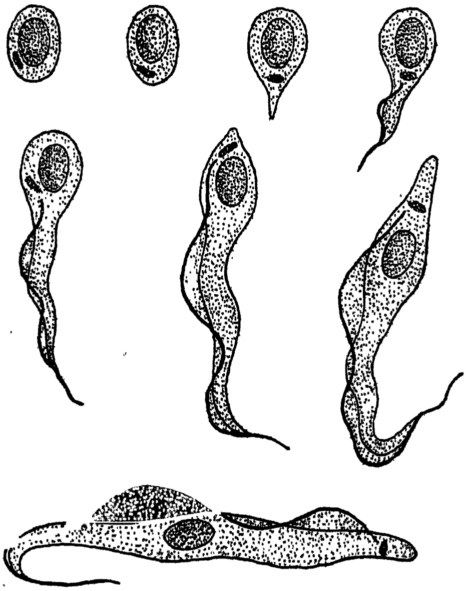
The post-flagellate or latent bodies vary in size (figs. 2–16, 22). This variation is due to two causes: (1) the formation of non-flagellate parasites from trypanosomes of different breadths, and (2) the occasional division of large post-flagellate forms by binary fission, an example of the simplest schizogony (fig. 23). This fission, so far as my researches go, is infrequent in the case of *T. gambiense* and *T. rhodesiense*. It has been observed that division of flagellate trypanosomes may immediately precede the formation of latent bodies.

Broad forms of *T. rhodesiense*, with posterior nucleus, may form relatively large latent bodies. As the nucleus is at or near the posterior (non-flagellar) end of the parasite, there is little of the body discarded in that region when rounding occurs. The kidney shape of the nucleus of some specimens of *T. rhodesiense* is seen in their latent bodies (fig. 27), and the nucleus lies to one side of the rounding body (figs. 17, 18). It is not easy, however, to differentiate between the latent bodies of *T. rhodesiense* and *T. gambiense*.

Moore and Breinl describe a stainable band or black line connecting the nucleus and blepharoplast of certain specimens of *T. gambiense* at or near the maxima. An "interaction" takes place between the blepharoplast and nucleus. After this the formation of latent bodies proceeds. During the researches now recorded, there was no good evidence found in support of Moore and Breinl's views. The stainable band was seen in some stout (probably old) parasites, and in parasites at periods near the death of the host. It is possible, as Swellengrebel (1908) suggested, that the stainable line is a form of degeneration. However, on the exact significance of the stainable band seen in such trypanosomes I prefer not to pronounce a definite opinion at present.

The Metamorphosis of Latent Bodies into Trypanosomes.

This process was observed in life on several occasions, though the complete passage from a rounded body to a fully flagellate moving trypanosome was only rarely seen (three times), for it is difficult to imitate precisely the natural conditions favourable to such a metamorphosis. However, by taking rounded bodies, usually obtained from the spleen of an infected rat, in a little physiological salt solution, and adding thereto an equal quantity of fresh (normal, uninfected) rat's blood, some of the rounded bodies were seen on a warm stage (25° to 35° C.) to grow, each becoming larger and sending out a process (text-fig. 2). This pseudopodium-like process lengthens, and a flagellum is formed from an area close to the blepharoplast (kinetonucleus). At this stage the parasite forms at its drawn-out anterior end an undulating membrane along the edge of which the flagellum lies and the organism somewhat resembles a Crithidia. This transitory stage may be termed the crithidial stage. The organism grows and the blepharoplast passes posterior to the nucleus, and the trypaniform stage is assumed.



TEXT-FIG. 2 represents diagrammatically the metamorphosis of a rounded, latent or non-flagellate parasite into a flagellate trypanosome (*T. gambiense*). The rounded bodies, obtained from the spleen of an infected rat, were placed in warm, fresh, uninfected rat's blood and watched under the microscope. The total time taken for the metamorphosis was about 1 hour.

The evidence of stained preparations (figs. 32 to 40) fully supports this mode of formation of flagellate trypanosomes from non-flagellate latent bodies.

The latent bodies, which are the post-flagellate stages of one generation of trypanosomes, become the pre-flagellate stages of the succeeding generation of trypanosomes. Such pre-flagellate, Crithidia-like parasites in various stages of metamorphosis may be seen in the peripheral blood of the host (figs. 32, 34 to 42), when the parasites are increasing in numbers therein.

A basal granule (blepharoplast of Minchin) is seen at the base of the flagellum of some stained flagellating parasites (fig. 37).

It may be added that in dealing with an isolated case of an intermediate stage of a parasite between the flagellate and the rounded body, it is sometimes difficult to determine whether the given stage is pre-flagellate or post-flagellate, that is, whether the given parasite is proceeding in development towards the flagellate stage or away from it towards the rounded body.

The Significance of the Non-flagellate or Latent Forms of Trypanosomes. Inoculation with Latent Bodies produces Trypanosomiasis.

R. Ross and D. Thomson (1910) report periodic variation in the numbers of the trypanosomes found in the blood of a patient W. A., suffering from Rhodesian Sleeping Sickness. Fantham and J. G. Thomson (1910) report similar periodic variation in the number of the parasites in the peripheral blood of sub-inoculated animals (rats, guinea-pigs, and rabbits). During the periods of decrease of the parasites in the peripheral blood, I find that latent (non-flagellate) bodies are present in relatively large numbers in the internal organs of the host. The latent bodies are formed at or near the period of maximum increase of the trypanosomes in the peripheral blood. The latent bodies are especially numerous in the spleen and bone marrow on the downward slope of the curve representing the numbers of the parasites in the peripheral blood of the host. Change of the latent forms into trypanosomes takes place on the rise or upward slope of the curve.

There is, of course, a mutual action and reaction of the host and the parasite, the resistance of the host probably being greatest when the flagellate trypanosomes within it are beginning to decrease, thus helping to bring about the assumption of the rounded form by many of the flagellates, so that latent or resistant non-flagellate stages of the parasite are then numerous.

The occurrence of latent bodies also helps to explain the successful inoculation of animals with trypanosomiasis when no flagellates can be found in the blood inoculated from a previously infected animal. Although it might be urged that flagellate trypanosomes, in numbers too few to recognise, may actually be present in the infected blood inoculated, yet it is possible to inoculate only latent, non-flagellate bodies, and give the inoculated animal trypanosomiasis. In other words, persistent infectivity in the case of trypanosomes is explained by rounded bodies.

I have performed this experiment (inoculation of latent bodies) on two occasions. In the first experiment a rat was inoculated with one drop of spleen-pulp (from Rat 7) mixed with a little sterile physiological salt solution, the mixture containing no flagellates. The inoculated rat developed trypanosomiasis on the 6th day, dying on the 12th day. The daily counts of this rat were as follows:—

Day ……	1	2	3	4	5	6	7	8	9	10	11	12
Parasites, per c. mm.	—	—	—	—	—	4	864	2196	4308	30,600	10,000	75,200

Secondly, a further experiment was tried, since it was considered that the parasites in one drop of spleen-pulp solution might be too numerous to count accurately, and that a few flagellate trypanosomes might be contained therein, for although a most minute search was made for flagellates in the fluid before inoculation, yet a very few flagellates might have remained undetected. Accordingly, in the second experiment, 1 cubic millimetre of spleen-pulp solution in sodium citrate was inoculated intraperitoneally. Careful examination of samples of the solution used, both fresh and stained, showed no flagellate trypanosomes, but many rounded bodies. The actual fluid used for inoculation also showed no flagellate trypanosomes when microscopically examined fresh. The rat thus inoculated developed trypanosomiasis, parasites being found on the 8th day, and it died on the 18th day. The daily counts of this rat are appended:—

Day…………	1	2	3	4	5	6	7	8	9	10	11
Parasites, per c. mm.	—	—	—	—	—	—	—	4	960	2800	12,000

Day…………	12	13	14	15	16	17	18
Parasites, per c. mm.	49,200	18,200	6400	2120	20,600	50,240	128,000

The two rats inoculated with non-flagellate, latent bodies of *T. rhodesiense* showed incubation periods rather longer (5 to 7 days) than usual (2 to 4 days) with rats inoculated with flagellate *T. rhodesiense.*

The occurrence of non-flagellate bodies in the life-cycle of the parasites of

Sleeping Sickness also explains recurrence of trypanosomiasis after it has apparently died out in an infected animal. In such cases the latent bodies are present in the host all the while in such organs as the spleen and bone marrow.

During these researches it has been found that flagellate trypanosomes (500,000 to 2,000,000 in number) inoculated into a rat or a guinea-pig can be detected in the peripheral blood-stream (in 1 cubic millimetre of blood) of the host for some 10 to 12 hours, or even 18 hours after inoculation. During and after this period, that is, during the incubation period of the parasite, a few rounded forms of trypanosomes can be found in a cubic millimetre of peripheral blood.

Biot (1910) writes of the "revivifying action" of physiological salt solution on trypanosomes (*T. lewisi*), especially in fluid from the liver of a rat dead 6½ days (kept in the cold, unopened). Biot does not explain the phenomenon. However, it is capable of explanation, for in the liver latent bodies of trypanosomes are present, which, under the relatively favourable environment of isotonic salt solution, flagellate and become typical trypanosomes.

In the treatment of trypanosomiasis by drugs, careful note must be taken of the occurrence of rounded, non-flagellate or latent forms of the parasite. A drug needs to be found which will either prevent the formation of rounded (latent) stages or disintegrate those latent bodies already formed. In this connection the work of B. Moore, Nierenstein, and Todd* on the combined use of salts of mercury and arsenic should be considered.

Note on the Degeneration of Trypanosomes.

All flagellate trypanosomes do not become rounded and form latent, non-flagellate bodies, but some of them degenerate and die. The degeneration may take various forms—some become (α) somewhat irregular and almost amœboid with pale-staining cytoplasm and vacuoles (figs. 44, 45), others (β) exhibit chromatolysis, wherein the nucleus becomes poor in chromatin and chromatoid granules occur in the cytoplasm (figs. 46—49), while others (γ) exhibit marked vacuolation (fig. 50). Such degenerating forms may be seen in various internal organs of the host, such as the lungs and spleen, especially during the period of formation of latent bodies.

It is also very probable that some of the latent bodies themselves die and do not flagellate, for some shrunken latent bodies, with undifferentiated contents, can be seen in the spleen.

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* 'Ann. Trop. Med. and Parasitol.' (1907), vol. 1, pp. 275—284.

Summary and Conclusions.

(1) Non-flagellate stages of trypanosomes, such as *T. gambiense* (Dutton) and *T. rhodesiense* (Stephens and Fantham), occur.

(2) These non-flagellate stages (" latent bodies " of Moore and Breinl) are especially found in the lungs, spleen, and bone marrow, during periods of decrease of trypanosomes in the peripheral blood of the host.

(3) They are in process of formation at or near the time when the trypanosomes are most numerous in the peripheral blood. The formation of latent bodies takes place especially in the lungs, and they collect in the spleen and bone marrow of the host.

(4) In the formation of non-flagellate stages, some of the cytoplasm and the flagellum of the trypanosome are disintegrated. The non-flagellate body contains the nucleus and blepharoplast (kinetonucleus) of the trypanosome.

(5) Non-flagellate (latent) bodies can be seen to grow and flagellate, turning into trypanosomes, when placed in fresh, warm, uninfected blood.

(6) Latent bodies of *T. rhodesiense,* inoculated into a rat, flagellate and produce trypanosomiasis.

(7) The non-flagellate (latent) bodies of trypanosomes (*T. gambiense* and *T. rhodesiense*) are the post-flagellate stages of one generation of trypanosomes and the pre-flagellate stages of the succeeding generation of trypanosomes.

(8) There is a life-cycle of trypanosomes (*T. gambiense* and *T. rhodesiense*) in Vertebrate hosts, comparable with those of Crithidia and Herpetomonas in the alimentary tracts of various Invertebrates. The latent (relatively resistant) stages of trypanosomes occurring in Vertebrates are separate from, and in addition to, stages of the parasite which may occur in the Invertebrate carrier (for example, Glossina).

(9) In the treatment of trypanosomiasis by drugs, careful note must be taken of the occurrence of rounded non-flagellate or latent forms of the parasite. A drug needs to be found which will either prevent the formation of rounded (latent) stages, or disintegrate those latent bodies already formed.

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EXPLANATION OF PLATE 15.

All figures were outlined with Abbé-Zeiss camera lucida, using 2-mm. apochromatic homogeneous immersion objective and compensating oculars 8 and 12 of Zeiss. Magnification 2000 diameters approximately, except where otherwise stated.

The chief stains used were iron hæmatoxylin, Giemsa, or Romanowsky after wet fixation.

The figures represent *T. rhodesiense*, as seen in rats, except where otherwise indicated.

Fig. 1 represents the formation of latent (non-flagellate) body from trypanosome in heart blood.

Figs. 2—5 represent latent bodies, with remains of trypanosomes around or near, from heart (fig. 4) and lungs.

Figs. 6, 7.—Oval latent bodies, each with nucleus and blepharoplast (kinetonucleus), from lung of rat and peripheral blood of guinea-pig respectively.

Figs. 8—16.—Various forms of latent bodies—from liver, heart, spleen, lung (figs. 8—11); of *T. gambiense* in peripheral blood of rat (fig. 12); from peripheral blood (fig. 13), lung, and heart respectively.

Figs. 17—20.—Rounding, herpetomonad-like forms, all from peripheral blood of guinea-pigs. Fig. 19 of *T. gambiense.*

Fig. 21.—Broad trypaniform parasite from blood of guinea-pig.

Fig. 22.—Latent form of *T. gambiense* from lung of rat.

Figs. 23, 24.—Possible division forms of latent bodies of *T. rhodesiense* from heart and lungs of rats. Fig. 24 magnified 1350 diameters. These forms are rare.

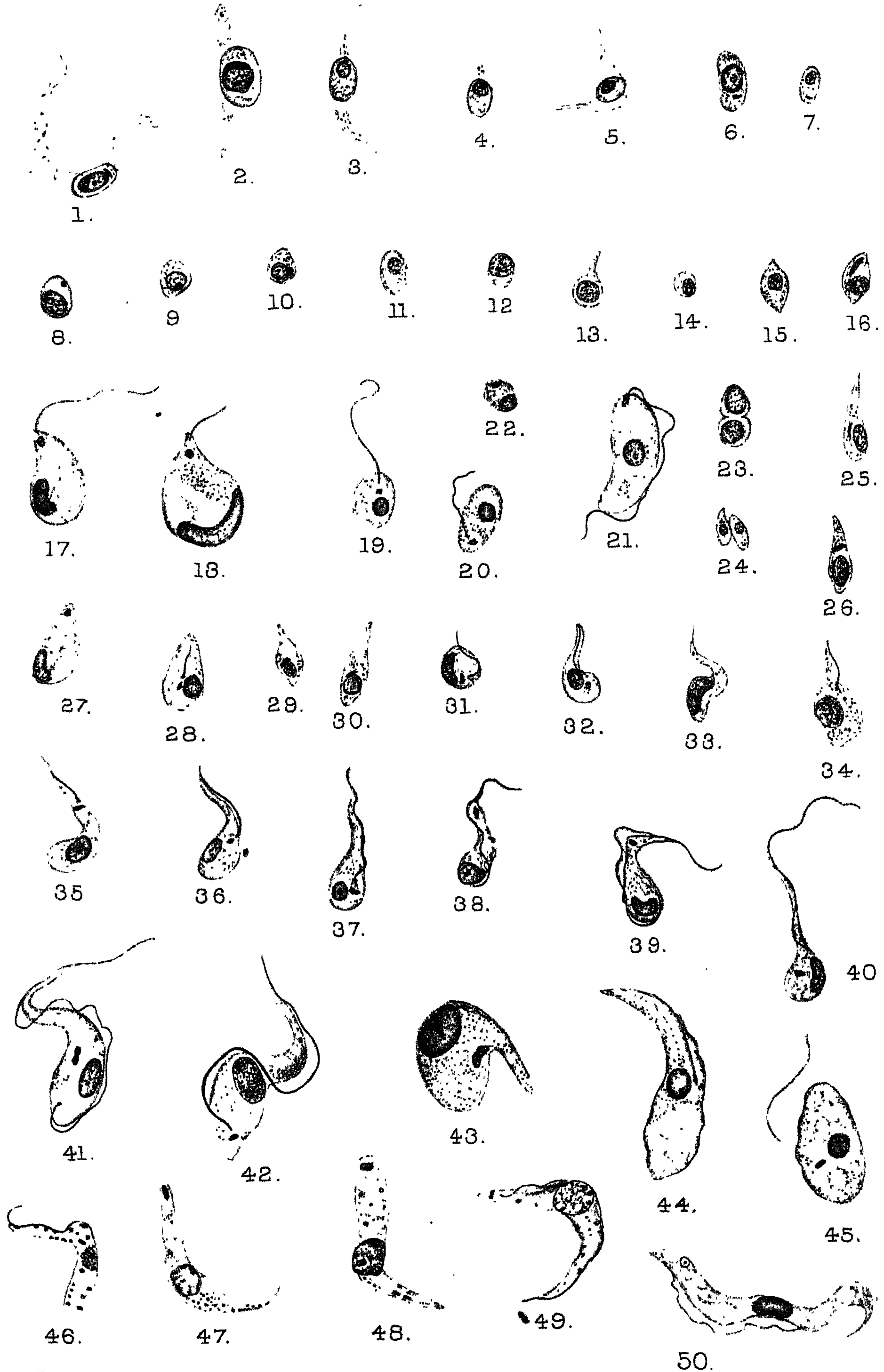
Figs. 25, 26.—Pyriform latent bodies, each with nucleus and blepharoplast. Fig. 25, of *T. rhodesiense* from blood of guinea-pig; fig. 26, of *T. gambiense* from blood of rat.

Figs. 27, 28.—Pyriform bodies of *T. rhodesiense* and *T. gambiense* (fig. 28) from peripheral blood of guinea-pig. In fig. 28 the flagellum is shown beginning to develop.

Figs. 29, 30.—Two pyriform non-flagellate forms from spleen of rat.

Fig. 31.—Round form of *T. gambiense*, with nucleus, blepharoplast and short flagellum along the edge of the body; from peripheral blood of rat.

Figs. 32—40.—Crithidia-like forms. Fig. 32, of *T. gambiense* from blood of rat; figs. 33—35, of *T. rhodesiense* from lung (fig. 33) and peripheral blood of rat; figs. 36—38, of *T. gambiense* from blood of guinea-pigs; figs. 39, 40, of *T. rhodesiense* from blood of guinea-pigs. In fig. 37 note the basal granule ("blepharoplast" of Minchin) at the root of the flagellum, forming the centrosome of the kinetonucleus.



H. B. Fantham, del.

Huth, Lith.r London

LATENT & OTHER FORMS OF
TRYPANOSOMA GAMBIENSE & T. RHODESIENSE.

Fig. 41 represents an almost trypaniform stage of *T. gambiense* from blood of guinea-pig. Note the peculiar position of the blepharoplast (kinetonucleus).

Fig. 42 represents a stout trypaniform parasite from heart blood of rat.

Fig. 43 shows peculiar, large rounding form (from blood of a rat), exhibiting signs of division of its nucleus and blepharoplast.

Figs. 44, 45.—Slightly irregular parasites, very pale staining and vacuolated. Degenerating, as seen in detachment of flagellum, etc.

Figs. 46—49.—Degenerating trypanosomes, exhibiting chromatolysis, all from lungs of rats. Fig. 46 of *T. gambiense*, magnified 1350.

Fig. 50.—Degenerating *Trypanosoma gambiense*, showing vacuolation, pale-staining cytoplasm and beginning of disintegration.

Experiments on the Treatment of Animals Infected with Trypanosomes, by means of Atoxyl, Vaccines, Cold, X-rays, and Leucocytic Extract; Enumerative Methods Employed. (Preliminary Note.)

By Prof. Major R. Ross, C.B., F.R.S., and J. G. Thomson, M.A., M.B., Ch.B.

(Received October 15,—Read December 8, 1910.)

These experiments were conducted with funds given by Sir Edwin Durning-Lawrence, Bart., for the purpose of testing the effect of cold on disease, and were suggested by Major R. Ross as a part of the studies made in connection with a case of Sleeping Sickness reported upon in an accompanying paper by him and Dr. David Thomson. Although many researches have been made on the effect of atoxyl and other drugs, we believe that these are the first in which that effect has been measured by regular daily counts of the parasites by measured thick film methods.

I. *Atoxyl.*

From a study of the patient W. A. at the Royal Southern Hospital by Major R. Ross and D. Thomson, it will be noted that atoxyl failed to be of any marked benefit to the patient, and in the doses administered there was no noticeable trypanocidal action. Various doses of atoxyl are recommended in treatment of human trypanosomiasis, and the patient (W. A.) received 4 grains as a maximum dose. It was impossible in this case to push the drug further, as the patient quickly showed signs of the toxic action of the drug.

Louis Martin and Henri Darré* believe that atoxyl can cure light forms of the disease, but that it is often necessary to give large doses, namely, 1 gramme, which imperil vision. These authors, however, still admit that permanent cure is a doubtful matter, and Sir David Bruce says that so far as we know the death-rate from Sleeping Sickness seems to be 100 per cent.

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* 'Bulletin of Sleeping Sickness,' 1910, vol. 2, No. 18.

In the experiments made by us we subinoculated rats with the Rhodesian strain of trypanosomes from the patient W. A. at the Royal Southern Hospital, in order that we might determine the effect of atoxyl on this particular form of trypanosomiasis, which, as will be seen from the accompanying paper on enumerative methods in untreated animals (Fantham and J. G. Thomson),* was of great virulence.

Eight rats were subinoculated with the Rhodesian strain from the patient (W. A.) at the Royal Southern Hospital. Four were treated with small doses of atoxyl, three were treated with large doses, and one was untreated.

(1) *Small Doses of Atoxyl.*—The weight of four rats treated with small doses varied from 139 grammes to 256 grammes. Take the body weight of a man as being 70 kilogrammes, and we find that the doses we administered to these rats would be represented in man by doses varying between 4·55 and 25·14 grains.

Rats treated with above Doses of Atoxyl.

Rat.	Colour.	Weight.	Duration of life.	
		grammes.	days.	
34	Piebald	139	27	Animal house.
35	"	145	26	Cold chamber.
36	White	251	27	"
37	"	256	8	Animal house.

Conclusions.

Small repeated doses of atoxyl in this strain, *i.e.* the Rhodesian strain, prolong the life of the host, probably because of the tonic action of the atoxyl. Two of the above rats treated with atoxyl lived 27 days, and one lived 26 days. The last only lived 8 days, but, unfortunately, this animal contracted pneumonia. When we compare this with the life of the untreated animals inoculated with this strain we find that in 22 rats inoculated, the longest life recorded is 18 days, and the average life of the 22 controls was 11·3 days (Fantham and J. G. Thomson). Atoxyl, therefore, in small doses, undoubtedly prolonged the lives of Rats 34, 35, and 36.

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* 'Roy. Soc. Proc.,' B, vol. 83, pp. 206—211.

Small repeated doses of atoxyl in this strain of trypanosomes actually stimulate division of the parasites. This conclusion is arrived at by the fact that in Rats 35 and 36 the number of parasites increased with great rapidity in the peripheral blood, and examination of the smears showed numerous trypanosomes in process of division. Many were found dividing into four.

It is interesting to note that the animal which died of pneumonia was living in the animal house. Rats 35 and 36 were treated in the cold chamber. As an explanation of the above, namely, the prolonged life of the animal and the rapid multiplication of the trypanosomes, we would venture to suggest the well known tonic action of arsenic when given to man in small therapeutic doses. In small repeated doses arsenic in man, both in health and disease, increases the strength, weight, and appetite, and we would argue that small doses of atoxyl in rats raised the natural *body* resistance.

In Rat 34, which lived 27 days, treated with small doses of atoxyl, we find that the period between the crests of the waves in a graph representing the numbers of trypanosomes was certainly larger than in those of the untreated animals of this same strain. Here two high crests occurred in the graph corresponding to intervals of 5 days and 10 days respectively.

This may probably be explained by the action of the atoxyl in small repeated doses raising the resistance of the host. The doses given to this animal would represent in man approximately 8·38 grains, 16·76 grains, and 25·14 grains.

We find that Gies* treated young rabbits with arsenic and found that those treated weighed more, had larger bones and more developed muscles than the untreated controls. These facts would explain the prolonged life of the animal, and we could at the same time attribute the increased activity in division of the trypanosomes to the small doses of atoxyl acting as a tonic to the trypanosome cells.

(2) *Large Doses of Atoxyl.*—We now treated three rats with large doses of atoxyl. In this we were guided by the excellent work of Dr. Wolferstan Thomas in his investigations on the action of drugs in trypanosomiasis. We gave 0·5 c.c. of a 5-per-cent. solution of atoxyl subcutaneously, and we caused the trypanosomes to disappear from the blood of one rat in 24 hours. In this rat the weight was 202 grammes, and if we calculate this dose to the body weight of a man, it would be necessary to give over 130 grains of atoxyl to a patient weighing 70 kilogrammes.

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* Cushny's 'Pharmacology.'

The trypanosomes at time of injection of the atoxyl were 21,736 per cubic millimetre of peripheral blood, and they all disappeared and were absent from the peripheral circulation for four days, when they suddenly reappeared (1600 parasites per cubic millimetre of blood).

This animal received a second injection of 0·5 c.c. of a 5-per-cent. solution of atoxyl, and the trypanosomes fell from 98,000 per cubic millimetre of peripheral blood to 89 per cubic millimetre. This animal lived 51 days.

In another rat weighing 129 grammes the trypanosomes also disappeared within 24 hours, but the animal evidently succumbed to the toxic action of the drug. The same dose was given. The third rat, weighing 113 grammes, succumbed to the toxic action of the drug. The control, weighing 238 grammes (untreated), lived 18 days. In large doses, therefore, atoxyl clears the trypanosomes from the peripheral blood, at least for a time. This is exactly in line with the discovery of Thomas and Breinl. These observers advocated the use of a large dose if treatment was to be successful.

Large doses of atoxyl are trypanocidal, but probably do not cure the animal, owing to the fact that atoxyl resistant forms round up in the spleen and bone marrow (Moore and Breinl).

Lastly we would like to state that in our opinion atoxyl is not a specific in this disease, but would appear to be as toxic to the body cells as to the trypanosomes themselves, and thus it is necessary to approach as near a lethal dose as possible if a permanent cure is to be expected. Small doses as given to man at present are in our opinion not trypanocidal, but might possibly prolong the life of the patient as it did in our rats.

II. *Vaccine Treatment.*

Previous work with vaccines and serums has given very indefinite results, and it seems that in animals little if any immunity is conferred by a previous attack of the disease (in *T. gambiense*).

We obtained a vaccine by inoculation of a rat with the Rhodesian strain. The disease was allowed to develop, and when the parasites were numerous in the peripheral blood the animal was killed.

The surface of the right auricle of the heart was seared with a hot needle in order to get a sterile portion, and through this the blood was drawn into a sterile pipette and mixed with normal saline. This mixture was placed for half-an-hour in an incubator at 55° C., and finally a little trikresol was added. A count was made of the parasites in 1 cubic millimetre before they were subjected to heat, and appropriate dilution was carried out with normal sterile salt solutions.

We now inoculated six rats with Rhodesian strain of trypanosomes, and incubation was allowed to take place. Three rats were treated with vaccine, and three were kept as controls.

Treated with Vaccine.		*Controls Untreated.*	
Rat 42	Lived 21 days.	Rat 14	Lived 12 days.
Rat 43	„ 9 „	Rat 15	„ 9 „
Rat 44	„ 11 „	Rat 16	„ 12 „

Here an interesting point can be noted. It will be seen that Rat 42 treated with vaccine lived 21 days, whereas Rats 43 and 44, also treated with vaccine, only lived 9 and 11 days respectively.

We would suggest that this is explained by the fact that the vaccine in the case of Rat 42 was administered in two doses of 10,000,000, with an interval of one day between the doses, whereas Rats 43 and 44 received two doses each of 12,000,000, no interval of a day being allowed.

In the case of Rat 42, we have the life prolonged in a remarkable manner. The longest life of our three controls was 12 days, and the average life of 22 untreated animals, infected with the same strain, was 11·3 days, where the longest life recorded was 18 days (Fantham and Thomson).

When a vaccine is administered during a natural rise of trypanosomes we have on the following day a rise in the number of trypanosomes in the peripheral blood, and this represents a negative phase. A second dose of vaccine ought therefore not to be administered at this period, as was done in Rats 43 and 44, or more harm than good may be accomplished. On the other hand, if we allow a day to intervene, as was done in Rat 42, we give the vaccine at a favourable period, the negative phase having passed off.

We admit, however, that much more extensive experiments will require to be undertaken, and would suggest that failure in vaccine treatment in trypanosomiasis until the present time has been caused by the vaccine being administered at the wrong time. Probably the best time to administer a vaccine is when the trypanosomes are low in numbers in the peripheral blood, and a second dose ought not to be administered until the negative phase has passed off (see R. Ross and D. Thomson).*

We have said that the trypanosomes increased in numbers in the peripheral blood after a subcutaneous injection of vaccine. This is in all probability to be explained by the hypothesis of Dr. H. C. Ross, who holds that the extracts of dead tissues stimulate cell division.†

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* 'Roy. Soc. Proc.,' B, vol. 83, pp. 187—205.

† 'Brit. Med. Journ.,' June 11, 1910.

III. *Treatment by Means of Cold.*

Here most interesting results have been obtained. Sir Edwin Durning-Lawrence, Bart., has taken great interest in the effect of cold in disease, especially tropical disease, and owing to his assistance these experiments have been made possible.

A chamber 12 feet long by 7 feet wide by 6¾ feet high was constructed, and this is cooled by a refrigerator made by Sir Alfred Haslam. The lowest temperature reached in our experiments was 20° F. The humidity of the atmosphere in this chamber was low and varied between 50 per cent. and 60 per cent., but this varied with the humidity of the outside atmosphere, as the door had to be opened several times daily for the purposes of observation.

Preliminary experiments were carried out in this cold chamber by Major Williams, and his results have been published in 'Annals of Tropical Medicine,' Liverpool, July, 1910.

Major Williams compares the atmosphere of the cold chamber to the climate of the interior of Canada. The patient, W.A., suffering from Sleeping Sickness contracted in Rhodesia, several times visited the cold chamber for treatment; but, unfortunately, at this time no counts were made of the parasites in the peripheral blood. We have, however, the evidence of the patient himself, who emphatically declared that he felt much better after being for some time in the cold. As the patient became worse, treatment had to be discontinued, owing to the fact that the patient was considered too ill to travel from the Hospital to the University; and we had to resort to treatment of subinoculated animals.

We used two strains of trypanosomes:—

(1) The Rhodesian strain (*T. rhodesiense*, Stephens and Fantham).

(2) The old laboratory strain of *T. gambiense*.

The essential difference between these two strains has been discussed in an accompanying paper on enumerative methods in untreated animals by H. B. Fantham and J. G. Thomson.

Five guinea-pigs were kept in the animal house, and four were kept in the cold chamber. Of these seven were subinoculated with the old laboratory strain of *T. gambiense*, and two were inoculated with the Rhodesian strain. The average incubation period of the controls in the animal house was four days, whereas the average incubation period of those treated in the cold was 13·5 days.

The average duration of life of the controls was 64·2 days, whereas the average life of those in the cold was 97·25 days. In addition to this the animals in the cold were livelier and took their food better than those in the animal house. We conclude that in our experiments the guinea-pigs had the incubation period delayed, and the life of the animal was prolonged in the cold.

We now experimented with rats. Again we used both strains of trypanosomes. Five rats were inoculated with the old laboratory strain of *T. gambiense*, three were treated in the animal house, and two were treated in the cold. Three rats were inoculated with Rhodesian strain. Two were treated in the animal house and one was treated in the cold. Here we find the average incubation period in the cold was 6·3 days, and the incubation in the animal house was 4·8 days. The average life in the cold was 16·3 days and the average in the animal house was 14·2 days. Daily enumerations were carried out in all the above animals. The conclusions to be drawn from the above are therefore in favour of treatment in the cold. The resistance of the animal body is evidently raised, for the incubation is delayed and life is prolonged.

In favour of the cold we have also the evidence of the patient W. A., who said he felt better in the cold chamber, and, lastly, all visitors to the cold chamber testified as to its bracing effect. What the physiological action of the cold is we are not prepared to state at present; but we certainly think that animals treated were beneficially affected, and that they were livelier. We think the cold dry atmosphere, which has been compared to the climate of Canada or Switzerland, raises the vitality of the whole animal organism and thus acted as a valuable therapeutic agent in treatment of Sleeping Sickness.

We did not cure the disease, but we were enabled to prolong life, and so we must regard the treatment as a valuable help in patients suffering from trypanosomiasis. Adding these results in animals to those previously reported by Major Williams, the *combined series of animals* experimented upon show very similar results.

IV. *Treatment with X-rays.*

A young piebald rat, weighing 77 grammes, was inoculated with the Rhodesian strain of trypanosomes. Here we have to thank Dr. Morgan, in charge of the Electrical Department at the Royal Southern Hospital, who kindly advised us in the administration of the rays. The exposures were given by Miss Wells, an experienced worker in electrical treatment, and we have to thank her for her kind assistance.

The rat lived 15 days, and we can therefore say that this animal's life was prolonged when compared with the average life of 22 controls, the average life of which was only 11·3 days.

In spite of five exposures, each of 20 minutes' duration, during which period the whole body of the rat was exposed to the direct action of the rays, the trypanosomes remained lively and increased steadily in numbers in the peripheral circulation. The rays were not therefore trypanocidal in the exposures given by us, but, curiously enough, the life of the animal was prolonged. The animal always seemed livelier during the exposure to the rays, and suffered no discomfort. There was no destruction of the trypanosomes, and this is comparable to the experiments made by one of us (R. R.) several years ago, who found that exposure of trypanosomes *in vitro* to the action of X-rays had no trypanocidal effect.

V. *Leucocytic Extract.*

This experiment was carried out by the suggestion of Dr. Moore Alexander, Pathologist to the Royal Southern Hospital, and he kindly advised us and supplied the leucocytic extract. A white rat, weighing 120 grains, was inoculated with the Rhodesian strain, and the disease was allowed to incubate. On the 12th day the trypanosomes numbered 89,000 per cubic millimetre, and we injected 0·5 c.c. of leucocytic extract. The leucocytes rose from 8,160 per cubic millimetre to 10,760 per cubic millimetre, and the trypanosomes rose from 89,000 to 220,800. The animal lived 14 days. We cannot, therefore, draw conclusions here, and much further work will require to be undertaken, before we conclude as to the value of leucocytic extract in trypanosomiasis.

Note upon the Examination, with Negative Results, of the Central Nervous System in a Case of Cured Human Trypanosomiasis.

By Frederick W. Mott, M.D., F.R.S.

(Received October 22,—Read November 3, 1910.)

This is the first case in which "a chance has occurred of examining a cured case of human trypanosomiasis *post mortem*," according to the statement of C. A. Wiggins, the Acting Principal Medical Officer of the Uganda Protectorate, who has kindly forwarded me the tissues for microscopic examination.

Summary of the History of the Case.—Narain Singh, a Sikh, belonging to the 4th K.A.R. (aged 30 at death), was found to be suffering from trypanosomiasis in June, 1905, and received treatment with inorganic arsenic. The previous history as far as ascertainable, compiled from extracts of reports and history sheets, is published in the full account, but, in brief, it may be assumed that the drug was given intermittently for 18 months or more, and pushed till toxic symptoms of neuritis, mental dullness, etc., rendered further energetic treatment impossible; trypanosomes were then no longer obtained by puncture of the glands. Unfortunately there is no note of lumbar puncture having been performed until a few months before death, therefore we do not know whether invasion of the sub-arachnoid space ever occurred either before or after the treatment. But it is probable that trypanosomes were never present in the cerebro-spinal fluid, for if they were, the symptoms of Sleeping Sickness would, in all probability, have come on, in spite of treatment by atoxyl. Sir David Bruce, in December, 1908 (that is three and a half years after the trypanosomiasis had been discovered), saw this man, and stated that he appeared to be in excellent health. A year later he was seen by Captains Hamerton and Bateman, who reported no symptoms of Sleeping Sickness. They made a very careful investigation of the blood, both by microscopic examination and by experimental injection into monkeys; the results were negative. In June, 1910, lumbar puncture was performed, and 17 c.c. of fluid withdrawn; the centrifuged fluid showed no lymphocytosis or trypanosomes; and injection of the fluid into a monkey was followed by negative results. The patient was attacked with pneumonia in August, 1910, and died three days after admission to the hospital. At the *post-mortem* examination grey hepatisation of the whole right lung was discovered. Beyond fibrosis and induration of the cervical and inguinal glands there was nothing noteworthy in the appearance of the organs and tissues. The brain was quite normal in appearance, and there was no excess of fluid.

It may be mentioned that this man contracted syphilis on July 27, 1906, and he was treated for some time with mercury, and a note on February 15, 1908, is as follows:—Condition the same as August 6, 1907, but no trypanosomes found in blood or glands, due to fact of taking mercury for syphilis.

Histological Examination.—Sections were prepared of portions of the cerebrum, cerebellum and medulla oblongata, by all the methods which I have previously adopted for the examination of the tissues of Sleeping Sickness cases. I found no trace of the characteristic meningeal and perivascular infiltration, nor of gliosis. Sections of the spleen, liver, and kidney were also examined with negative results.

I have shown that there is a parallelism between the intensity of the signs and symptoms of Sleeping Sickness and the diffuseness and intensity of the lymphatic perivascular infiltration with lymphocytes and plasma cells which is the main cause of the clinical phenomena.

It may be asserted that this case proves that human trypanosomiasis is curable, but it does not prove that Sleeping Sickness is curable, for I contend that the diagnosis of "Sleeping Sickness" can only be made when there is proof that the trypanosomes have invaded the sub-arachnoid space. The case emphasises the importance of early diagnosis of the infection and the value of early energetic treatment by organic arsenic preparations. How far the administration of mercury assisted to complete the cure is uncertain.

UGANDA PROTECTORATE.

Death Report.

Entebbe Station, August 17, 1910.

Name.	Nationality.	Age.	Sex.	Date of admission.	Date of death.	Disease for which admitted.	Cause of death.	Place of death.
Narain Singh, No. 4662 I.C. 4th K.A.R.	Sikh	30	Male	3.8.10	6.8.10	Bronchitis	Pneumonia	Indian Contingent, 4th K.A.R. Hospital.

Previous history as far as ascertainable, compiled from extracts of reports and history sheets:—

22.6.05. Trypanosomiasis. Received treatment inorganic arsenic.
4.12.06. Gland puncture. Trypanosomes +.

Four injections 2 c.c. atoxyl 20 per cent. December 6, 7, 16, and 17.

18.12.06. Glands trypanosomes +.

28.12.06. Glands trypanosomes −. Atoxyl 20 per cent., 2 c.c.

29.12.06. Repeated atoxyl 20 per cent., 2 c.c.

30.1.07. Glands trypanosomes −.

8.2.07. Glands trypanosomes −.

Condition.—Arsenical neuritis. Drowsy looking. Rheumatic pains.

6.8.07. Condition far from satisfactory. Irregular fever, rapid pulse. Dull and stupid. Cervical glands enlarged. Trypanosomes not found in glands since March, 1907. Energetic treatment with arsenic impossible owing to symptoms of poisoning rapidly appearing.

15.2.08. Condition the same 6.8.07, but no trypanosomes found in blood or glands, due to the fact of taking mercury for syphilis (contracted 27.7.06).

16.12.08. *General Condition.*—Strong, well nourished. No tremors. Reflexis normal.

Gland puncture and blood examination trypanosomes −.

Differential count. Lymphocytes 70 per cent.

Some tendency to drowsiness when unoccupied.

Last treatment, two injections kharsin, May, 1908.

18.12.08. Proceeded to Mpumu to do duty with the Royal Society's Sleeping Sickness Commission. Report by Sir David Bruce—"This man appears to be in excellent health." Returned to Entebbe.

16.12.09.—Report by Captains Hamerton and Bateman, R.A.M.C., Sleeping Sickness Commission, Mpumu—"No symptoms of Sleeping Sickness."

Blood Examination.—Examined peripheral blood. Negative results 14 occasions.

Examined centrifuged peripheral blood. Results negative.

Examined blood from median basilic vein. Results negative.

Examined blood from median basilic vein centrifuged. Results negative.

16.12.09. 4 c.c. blood from median basilic injected into monkey.
29.12.09. 5 c.c. " " " "
1st monkey examined 15 times. Results negative.
2nd " " 12 " " "

13.6.10. Lumbar puncture 17 c.c. Cerebro-spinal fluid abstracted centrifuged. Trypanosomes −. No excess white cells. 12 c.c. injected into monkey. Monkey examined twice weekly over one month. Results negative.

General habits.—Intemperate. Had gonorrhœa and syphilis.

Post-mortem *appearances* 14 *hours after Death.*

Body of lean but in no way emaciated man.

Externally.—Cervical glands—small, hard, and shotty. Inguinal glands—small, hard, and shotty.

Chest.—Parietal and visceral pleura adherent right side, due to recent pleurisy.

Right Lung.—Weight 58 ozs. On section the whole lung was in a state of grey hepatisation.

Left Lung.—Weight 14 ozs. Pleura and lung substance normal.

Heart.—Weight 12 ozs. Right heart distended with blood clot. All chambers and valves normal. The glands at the root of lungs and around bronchi and trachea were enlarged and soft, and some were purulent.

Microscopic.—Examination of this pus showed organisms resembling pneumococci.

Liver.—Weight 89 ozs. Enlarged and congested. On section, soft and friable. Probably some fatty change. No obvious fibrotic changes.

Portion preserved for microscopic examination in England.

Spleen.—Weight 14 ozs. Feels hard to the knife on section. Pale appearance and excess of fibrous tissue on cutting open.

Portion preserved for microscopic examination in England.

Kidneys.—Right, weight 5 ozs. Left, 6 ozs. Both were swollen, the kidney substance bulging from the capsule when cut into.

Both capsules were a little adherent.

Cortices undiminished. The whole kidney substance appeared a little paler than normal.

Portions preserved for microscopic examination in England.

Pancreas and Suprarenals.—Normal.

Stomach.—Slightly dilated. No gastritis. Some *post-mortem* staining.

Intestines.—Normal. Mesenteric and retroperitoneal glands not enlarged.

Brain.—Calvaria normal. Weight of brain, 52 ozs. No thickening of membranes. Brain substance normal. Ventricles normal in size and no excess of fluid.

Portions of cerebral cortex and cerebellum preserved for examination in England.

(Signed) H. B. Owen,
Medical Officer, Civil Hospital, Entebbe.

Auto-Agglutination of Red Blood Cells in Trypanosomiasis.

By Warrington Yorke, M.D.

(Communicated by Major Ronald Ross, C.B., F.R.S. Received November 2, 1910,—Read January 19, 1911.)

From the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine.)

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Introduction.—It is now a well recognised fact that the erythrocytes in fresh preparations of the blood of Sleeping Sickness cases and animals infected with trypanosomiasis frequently exhibit a more or less marked degree of agglutination. Attention was first drawn to this phenomenon in 1898 by Kanthack, Durham and Blandford,* who found that the red blood cells of animals infected with nagana, instead of forming rouleaux, tended to clump together into masses and to lose their outlines.

More recently Christy† (1904), Dutton and Todd‡ (1905), Martin, Lebœuf and Roubaud§ (1906–8), and others have described a similar condition in fresh preparations of the blood of patients suffering from Sleeping Sickness.

Dutton and Todd,‖ referring to this condition of the blood, wrote: "Only once have we had the opportunity of observing a patient (European) from whose blood trypanosomes, once present, have finally disappeared. In this instance auto-agglutination of the red cells disappeared with the parasites.

Later, it has been noted by many investigators¶ that the phenomenon gradually disappears in animals after the successful drug treatment of the disease.

In spite of the fact that auto-agglutination of the red cells in trypanosomal infections has attracted so much attention, very little work appears to have been done with a view to determining the nature of the changes in the blood which lead to its manifestation. Most of the workers have contented themselves with simply recording the presence of the phenomenon. So far as have been able to ascertain from a search of the literature, only two authors mention any details regarding the mechanism of its production.

Kanthack, Durham and Blandford stated that the serum of the blood of animals exhibiting auto-agglutination, when added to normal blood of the same species of animal, caused the red corpuscles to clump together. On the other hand, at a recent meeting of the Society of Tropical Medicine and Hygiene, in the discussion following Dr. Bagshawe's paper on "Recent

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* "On Nagana or Tsetse Fly Disease," 'Roy. Soc. Proc.,' 1898, vol. 64, p. 100.

† "Sleeping Sickness," 'British Medical Journal,' 1904, p. 1456.

‡ "Gland Puncture in Trypanosomiasis," 'Memoir of the Liverpool School of Tropical Medicine,' 1905, No. 16, p. 99.

§ 'Rapport de la Mission d'Études de la Maladie du Sommeil au Congo Français,' 1906—8, p. 281.

‖ *Loc. cit.*

¶ Thomas and Breinl, "Pathology and Treatment of Trypanosomiasis," 'Memoir of the Liverpool School of Tropical Medicine,' 1905, No. 16.

Mensil, Nicolle, and Aubert, "Recherches sur le Traitement des Infections Expérimentales à *T. gambiense*," 'Annales de l'Institut Pasteur,' 1907, vol. 21, p. 1.

Laveran and Thiroux, "Sur le Traitement des Trypanosomiases," 'Bulletin Soc. Path. Exot.,' 1908, p. 28.

Martin and Darré, "Sur les Symptômes Nerveux du Début de la Maladie du Sommeil," 'Bulletin Soc. Path. Exot.,' 1908, p. 15.

Advances in our Knowledge of Sleeping Sickness," Breinl* remarked that with regard to its (auto-agglutination) mechanism he had not, up to the present, been able to arrive at any definite conclusions. All his attempts to isolate a hæmagglutinin had failed, and he had not been able to demonstrate either an iso- or an auto-agglutinin. Dr. Nierenstein† had shown that in trypanosomiasis a definite increase of the acidity of the blood occurred. This was most probably due to an increase of the amino-acids in the blood, and Breinl and Nierenstein inclined to the view that auto-agglutination was caused by this excess of amino-acids. Unfortunately, they had not so far been able to find a definite proof of the correctness of this conception.

The results obtained by these investigators are thus somewhat conflicting, for whereas Kanthack, Durham, and Blandford found an iso-agglutinin in the serum of certain animals infected with *T. brucei*, Breinl was unable to demonstrate the existence of either an auto- or an iso-agglutinin.

Examination of Serum of Infected Animals for Auto-agglutinin.—Having under observation a number of animals, the blood of which when examined in cover-slip preparations exhibited the phenomenon of auto-agglutination to a greater or less degree, it was decided to perform experiments with a view to investigating the mechanism of its production.

Technique.—The blood was collected in a solution containing 1 per cent. sodium citrate and 0·9 per cent. sodium chloride in distilled water. The blood was then centrifugalised and the corpuscles washed three times in normal saline solution. A 5-per-cent. suspension of the washed red cells was then made in normal saline. Another sample of the same blood was collected and allowed to clot and the serum subsequently freed from the clot by centrifugalisation.

Equal amounts of the serum and emulsion of red corpuscles were then mixed together and drawn up into a capillary tube which was placed vertically in the incubator at 37° C.

The results of such experiments may be briefly summarised. With the exception of a few cases in which there appeared to be a trace of agglutination they were all negative. Similar negative results were obtained when the sera were examined for the presence of iso-agglutinins and also when the serum was replaced by citrated plasma.

At first one was somewhat at a loss to account for these apparently conflicting results, as even the sera of animals where the cover-slip preparations exhibited the most marked auto-agglutination of the red cells gave invariably negative results when examined for auto- and iso-agglutinins by this method.

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* 'Trans. Soc. Trop. Med. and Hygiene,' 1909, p. 29.

† "Observations on the Acidity and Alkalinity of the Blood in Trypanosome Infections," 'Annals of Tropical Medicine and Parasitology,' 1908, p. 227.

Later it was observed that when the blood from one of the infected animals was allowed to flow from a vein of the ear into a watch-glass containing a small amount of citrated saline solution, the red cells quickly sank to the bottom in little clumps, producing in a marked degree the sandy appearance described by Dutton and Todd.* When, however, the watch-glass and salt solution were warmed to 37° C. and the blood dropped in as previously, this appearance did not result; the red cells remained suspended for a considerable time and only subsided gradually, as in the case of normal blood. So long as the temperature of the watch-glass and salt solution was kept at 37° C., no agglutination resulted, but as soon as the temperature was allowed to fall to about 18° C., the red cells ran together into clumps and the typical sandy appearance was obtained.

These observations served to indicate that temperature played an important *rôle* in the development of the phenomenon. Accordingly, the previous experiment was repeated, but on this occasion three sets of tests were made: the first were placed in the incubator at 37° C., the second were left at laboratory temperature (18° to 21° C.), the last were kept in the ice chest at 0° C. Even at the end of five minutes a certain degree of agglutination of the red cells was noticeable in some of the tubes which had been placed in the ice chest, whilst in 15 minutes the red cells in most of them were completely agglutinated, numerous clumps of various sizes being visible in the clear serum. The reaction was also distinct in many of the tubes kept at the laboratory temperature, but it was neither so marked, nor did it occur so quickly as in those subjected to the lower temperature. As before, no agglutination—or only occasionally a trace—was observable in the tests which had been placed in the incubator at 37° C.

A large number of similar experiments were subsequently performed with the blood of monkeys, donkeys, goats, dogs, rabbits, guinea-pigs, and rats infected with various strains of trypanosomes. As a rule, it was found that a marked degree of agglutination only resulted when the temperature of the mixture of serum and red cells was lowered. Very exceptionally slight traces of agglutination were also seen in the tests carried out at 37° C., but these could not be compared with the intensity of the reaction at low temperatures.

Quite frequently a well-marked auto-agglutination was found to occur, at 0° C., in the control tests made with the blood of normal animals. I shall return to this important point later.

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* *Loc. cit.*

Iso-agglutination.—A series of experiments were undertaken with a view to ascertaining whether the sera of those infected animals which possessed the property of agglutinating their own red cells were also capable of producing agglutination of the erythrocytes of other animals of the same species. In every case where auto-agglutination was present, iso-agglutination was found to occur when the serum of the infected animal was added to the red cells of another animal (either normal or infected) of the same species.

Analogous results were obtained with the blood of a case of human trypanosomiasis in Major Ross's clinic in Liverpool. Cover-slip preparations of the blood of this case exhibited a certain degree of auto-agglutination during the six months he was under observation. The following table gives the results of an examination of his blood, drawn 10 days before his death, for auto- and iso-agglutinins.

Table I.—Examination of Sleeping Sickness Serum for Auto- and Iso-agglutinin.

Equal volumes of serum and red blood cell suspension used.		Temp.	Result.
5 per cent. suspension of washed erythrocytes in normal saline solution.	Source from which serum was obtained.		
		° C.	
Sleeping Sickness.........	Sleeping Sickness......	37	No agglutination in 30 mins.
		18	Marked „ 30 „
		0	Complete „ 10 „
Normal individual A ...	Sleeping Sickness......	37	No agglutination in 30 „
		18	Complete „ 30 „
		0	„ „ 10 „
Normal individual B ...	Sleeping Sickness......	37	No agglutination in 30 „
		18	Marked „ 30 „
		0	Complete „ 10 „
Normal individual C ...	Sleeping Sickness......	37	No agglutination in 30 „
		18	Marked „ 30 „
		0	Complete „ 10 „
Normal individual C ...	Normal individual C	37	No agglutination in 30 „
		18	„ „ 30 „
		0	„ „ 30 „
Normal individual C ...	Normal individual B	37	No agglutination in 30 „
		18	„ „ 30 „
		0	Marked „ 30 „

Spontaneous Agglutination of the Red Cell Suspensions.—On rare occasions it was found that the 5-per-cent. suspension of red cells which had been washed three times in large volumes of 0·9-per-cent. sodium chloride solution underwent a spontaneous agglutination in the entire absence of serum. Indeed, in one or two instances, where the animals exhibited an extreme degree of auto-agglutination, some difficulty was experienced in obtaining an even suspension of the erythrocytes. The probable explanation of this spontaneous agglutination is that it was due to the absorption of agglutinin from the plasma by the red cells immediately after the blood was shed into the cold citrated saline solution.

This difficulty was obviated by collecting the blood in warm citrate solution and then rapidly centrifugalising and decanting off the citrated plasma. The red cells were then washed thrice in warm normal saline solution. Suspensions prepared in this way exhibited no tendency to spontaneous clumping.

It might be mentioned in this connection that Klein* has succeeded in obtaining agglutinating solutions by grinding up with quartz sand the well-washed erythrocytes of certain animals (rabbit, dog, hen, and guinea-pig). These extracts sometimes agglutinated the red cells of other animals, and frequently also the erythrocytes of the same kind of animal, and even those of the same animal.

Absorption of Agglutinin by Red Cells.—Experiment. To one volume of the citrated plasma of Rabbit 896 (infected with *T. dimorphon*), which caused great agglutination when added to its own red cells and to those of normal rabbits, were added five volumes of the undiluted well-washed red cells of the same animal. The mixture was then divided into two equal portions, A and B. A was placed in the incubator at 37° C. and B in the ice chest at 0° C. At the end of three hours the extracted plasmas were separated from the red cells by centrifugalisation, and were examined for auto- and iso-agglutinins.

Table II.

Equal volumes of extracted plasma and erythrocyte suspension used. Temp. of experiment 0° C.		Result.
5 per cent. suspension of washed erythrocytes in normal saline solution.	Extracted plasma.	
Rabbit 896	A	Complete agglutination in 10 mins.
"	B	No agglutination in 60 mins.
Normal rabbit	A	Complete agglutination in 10 mins.
"	B	Slight agglutination in 60 mins.

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* "Beiträge zur Kenntniss der Agglutination rother Blutkörperchen," 'Wien. Klin. Woch.,' 1902, No. 16, p. 413.

The plasma which had been in contact with the red cells at 0° C. had almost completely lost its agglutinating action, whilst the other portion, B, which had been placed in the incubator, had retained its auto- and iso-agglutinins intact.

The following observation furnishes additional proof of the capacity of erythrocytes to absorb hæmagglutinin in the cold. Specimens of the blood of a number of infected animals were collected in sterile tubes and placed immediately in the ice chest to clot. After six hours the sera were separated from the clots by centrifugalisation and were examined for auto- and iso-agglutinins. In several cases the results were negative, no auto- or iso-agglutination being observed. In other instances the red cells were found to be clumped to a greater or less degree. The amount of agglutinin present in these sera was then compared with that occurring in the citrated plasma of the same animals which had been separated from the red cells at 37° C. The following procedure was adopted:—The serum and plasma were diluted with gradually increasing amounts of 0·9-per-cent. NaCl solution, and the degree of dilution observed at which they no longer caused complete agglutination of a given volume of the red cell suspension in a stated time.

The results obtained with one of the animals (Rabbit 1035, infected with *T. brucei*) are given in tabular form. It is to be observed that there was at least five or six times as much agglutinin in the plasma which had been separated from the red cells at 37° C. as in the serum obtained from blood which had been allowed to clot in the ice box.

The sera of the other animals all showed a considerable deficiency in agglutinin as compared with that present in the plasma of the same animals.

When examining the blood for auto-agglutination it was found that the strongest reactions were obtained by dropping the blood into a very small quantity of warm citrated saline solution, and then separating the plasma from the red cells as speedily as possible with the centrifuge. A perhaps even more satisfactory method, and one in which the dilution of the plasma by the citrate is avoided, is to use the defibrinated plasma obtained by shaking the blood at a sustained temperature of between 37° and 40° C. in a bottle containing a few glass beads.

Well marked agglutination was frequently observed when the plasma obtained in this manner was added to a 5-per-cent. suspension of red cells, whilst either a negative or only slightly positive result was obtained with the serum derived from blood which had clotted at laboratory temperature or in the ice chest.

Reversibility of the Reaction.—In view of the fact that red cells absorb agglutinin to a much greater extent at low temperatures and only slightly

Table III.—Diminution of Amount of Auto- and Iso-agglutinin in Serum obtained from Blood which was allowed to Clot at 0° C.

Equal volumes of red cell suspension and diluted serum or plasma used. Temp. of experiment 0° C.		Result.
5 per cent. suspension of washed red cells of Rabbit 1035 (infected with *T. brucei*) and of normal rabbit.	Serum of Rabbit 1035 obtained by allowing the blood to clot at 0° C. Plasma of Rabbit 1035 separated from the red cells at 37° C.	
Rabbit 1035	Undiluted serum	Complete auto-agglutination in 15 mins.
	„ plasma	„ „ 10 „
Normal rabbit	„ serum	Complete iso-agglutination 10 „
	„ plasma	„ „ 10 „
Rabbit 1035	Serum diluted with an equal volume of 0·9 per cent. NaCl solution	Complete auto-agglutination in 20 „
	Plasma „ „ „	„ „ 10 „
Normal rabbit	Serum „ „ „	Complete iso-agglutination 10 „
	Plasma „ „ „	„ „ 10 „
Rabbit 1035	Serum diluted with 2 volumes of 0·9 per cent. NaCl solution	Complete auto-agglutination in 30 „ *
	Plasma „ „ „	„ „ 15 „
Normal rabbit	Serum „ „ „	Complete iso-agglutination 15 „
	Plasma „ „ „	„ „ 10 „
Rabbit 1035	Serum diluted with 3 volumes of 0·9 per cent. NaCl solution	Partial auto-agglutination in 30 „
	Plasma „ „ „	Complete „ 30 „
Normal rabbit	Serum „ „ „	Complete iso-agglutination 20 „ †
	Plasma „ „ „	„ „ 20 „
Rabbit 1035	Serum diluted with 4 volumes of 0·9 per cent. NaCl solution	Trace auto-agglutination in 30 mins.
	Plasma „ „ „	Complete „ 30 „
Normal rabbit	Serum „ „ „	Very marked iso-agglutination 30 „
	Plasma „ „ „	Complete „ 20 „
Rabbit 1035	Serum diluted with 6 volumes of 0·9 per cent. NaCl solution	No agglutination in 30 mins.
	Plasma „ „ „	Complete auto-agglutination 30 „
Normal rabbit	Serum „ „ „	Slight iso-agglutination 30 „
	Plasma „ „ „	Complete „ 20 „
Rabbit 1035	Serum diluted with 9 volumes of 0·9 per cent. NaCl solution	No agglutination in 30 „
	Plasma „ „ „	Complete auto-agglutination 30 „
Normal rabbit	Serum „ „ „	No iso-agglutination 30 „
	Plasma „ „ „	Complete iso-agglutination 20 „
Rabbit 1035	Plasma diluted with 12 volumes of 0·9 per cent. NaCl solution	Complete auto-agglutination in 30 „ ‡
Normal rabbit	„ „ „ „ „	„ iso-agglutination 30 „
Rabbit 1035	Plasma diluted with 14 volumes of 0·9 per cent. NaCl solution	Partial auto-agglutination in 30 „
Normal rabbit	„ „ „ „ „	Complete iso-agglutination 30 „ §
Rabbit 1035	Plasma diluted with 19 volumes of 0·9 per cent. NaCl solution	No agglutination in 30 „
Normal rabbit	„ „ „ „ „	Slight iso-agglutination 30 „

* Denotes the end point of the auto-reaction of serum.
‡ „ „ „ plasma.

† Denotes the end point of iso-reaction of serum.
§ „ „ „ plasma.

at higher temperatures (37° to 40° C.), the question arises as to whether the absorption of agglutinin belongs to the group of reactions which have been designated "reversible."[*] In other words, will raising the temperature of the agglutinated masses of red cells cause the clumps to disintegrate into their corpuscular elements?

Experiment.—One volume of red corpuscles of Rabbit 1022 (infected with *T. gambiense*) was added to 20 volumes of the defibrinated plasma of the same animal. After an hour's sojourn in the ice chest complete agglutination of the erythrocytes was found to have occurred. After stirring the clumps up thoroughly with a glass rod a small drop of the suspension was placed on a cover-slip and a hanging drop preparation made. On examining with the microscope large masses of agglomerated red blood cells and also considerable rouleaux formation were seen. The mixture of clumped red cells and defibrinated plasma was now placed in the incubator at 37° C. In about 15 minutes the clumps were no longer visible, and the erythrocytes appeared to be evenly suspended throughout the fluid. A hanging drop preparation was made on a warm slide and cover-slip. As long as the temperature was maintained at 37° C., there was no tendency to agglutination. When the suspension was again cooled to 0° C., agglutination of the red cells reappeared after a few minutes.

From this and similar experiments it follows that the reaction is reversible, the phenomenon disappearing on warming and reappearing on cooling.

Auto-agglutination in the Blood of Normal Animals.—Attention has already been drawn to the fact that a certain amount of auto-agglutination was frequently observed in the control tests of normal blood. Some years ago Klein[†] found auto-agglutinin to be present in the serum of a number of normal horses.

Landsteiner[‡] demonstrated the existence of a similar substance in the blood of rabbits, horses, dogs, and cattle.

Other writers, on the contrary, deny the existence of auto-agglutinin in normal blood. Dudgeon[§] in a recent paper states that auto-agglutination does not occur in normal human blood.

It was decided to re-investigate this subject more fully, using the blood of considerable number of normal animals of different kinds.

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[*] Arrhenius, 'Immuno-Chemistry,' ch. 2.

[†] 'Beiträge zur Kenntniss der Agglutination rother Blutkörperchen," 'Wien. Klin. Woch. 1902, No. 16, p. 413.

[‡] "Ueber Beziehungen zwischen dem Blutserum und den Körperzellen," 'Münch. Med. Woch.,' 1903, No. 42.

[§] "On the Presence of Hæmagglutinins, etc., in the Blood obtained from Infectious and Non-Infectious Diseases in Man," 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 207.

Technique.—The blood was obtained from a convenient vein, and the defibrinated plasma separated from the red corpuscles at 37° C. in the manner already described. The red corpuscles were washed three times in warm saline solution, and finally a 5-per-cent. suspension made in 0·9-per-cent. sodium chloride solution. Equal volumes of the defibrinated plasma and red cell suspension were drawn up together into three fine pipettes which were then subjected to a temperature of 0°, 15°, and 37° C. respectively. The pipettes were kept in the vertical position, and the contents examined for auto-agglutination with the aid of a lens from time to time. It was found in the majority of cases that the test could not well be continued for longer than one hour, owing to the fact that in most cases the erythrocytes had subsided to a marked degree after the lapse of this period. At times the citrated plasma was substituted for the defibrinated plasma. The same precautions regarding temperature were taken, and only very small amounts of citrate solution (not more than a tenth of the volume of plasma) employed. The plasma obtained in this way frequently clotted, but the process was sufficiently retarded to permit of the previous separation of the red corpuscles. No appreciable difference between the agglutinating action of the defibrinated and citrated plasma was observed.

The results of this investigation of normal blood for auto-agglutinin may be summarised by stating that small quantities of auto-agglutinin were found to be present in the blood of rabbits (14), guinea-pigs (4), goats (3), dogs (2), horses (4), donkeys (2), monkeys (*Macacus rhesus*) (2), and *Cercopithecus callitrichus* (2). Sometimes, especially in goats and guinea-pigs, the amount present was exceedingly small, and considerable care was necessary to demonstrate its existence. In these cases a larger volume of serum was used in proportion to the amount of red cells and the reaction allowed to proceed for a longer period. It is to be observed that clumping of the erythrocytes only occurred in the tests carried out at low temperatures, and not in those subjected to a temperature of 37° C.

Relation of Auto-agglutinin of Normal Blood to that present in the Blood of Animals infected with Trypanosomes.—In this connection it may be remarked that in the blood of infected animals there exists a considerable excess of auto-agglutinin beyond that present in the blood of normal animals. It was found that diluting the defibrinated plasma of normal blood with twice its volume of normal saline solution usually sufficed to destroy its agglutinating action. On the other hand, it was often possible to dilute the infected plasma 15- or 20-fold, and still obtain complete agglutination of the erythrocyte suspension.

Effect of Heat on Auto-agglutinin.—Different portions of the defibrinated plasma of normal and infected animals were heated in a water bath to 58° C. and 70° to 72° C. respectively for 20 minutes. Heating to 58° C. was found not to destroy auto-agglutinin, whereas plasma which had been subjected to a temperature of 70° C. for 20 minutes had completely lost this property.

Significance of the Phenomenon in Trypanosomal Infections.—The question of the mechanism of production of auto-agglutination in trypanosomal infections is one which has frequently been discussed, but as yet no satisfactory explanation has been offered. With reference to this question it appears to me that two theories might be advanced to explain the development of an excess of auto-agglutinin in this disease.

It has long been recognised that the blood of men and the lower animals suffering from trypanosomiasis is frequently very anæmic. Both the percentage of hæmoglobin and the number of red corpuscles per cubic millimetre fall to a low level. This is particularly the case in the last stages of the disease. Conceivably auto-agglutinin might develop in the plasma as a result of auto-inoculation of an animal resulting from the destruction of its own erythrocytes.

There are, however, many considerations which operate against this view. In the first place I have found no constant relation between the development of anæmia and auto-agglutination of the red cells. By the aid of systematic hæmocrit examinations of the blood of recently infected animals it was observed that auto-agglutination was usually pronounced for a considerable period before any marked fall of the hæmocrit value had occurred. Secondly, a marked degree of auto-agglutination comparable to that occurring in trypanosomiasis has not been described in any other of the diseases in which anæmia is a distinctive feature. Dudgeon* examined the blood of 26 cases of anæmia due to various causes without finding a single example of auto-agglutination. It is doubtful, however, whether the technique adopted by Dudgeon is suitable for the recognition of small amounts of auto-agglutinin. Then again it is generally recognised as impossible to evoke the production of auto-bodies experimentally by inoculating an animal with its own tissues.

Experiment.—A rabbit was injected intraperitoneally with 10 c.c. of its own erythrocytes which had been laked with distilled water and the resulting solution made isotonic with sodium chloride. This injection was repeated after an interval of a week. No increase of auto-agglutinin was found to occur in the animal's blood.

Another possible explanation for the cause of the development of auto-agglutinin is that it is formed by the animal mechanism as a direct response to the stimulus of the pathogenic agent. In the consideration of this question it is necessary to inquire whether auto-agglutinin alone is present in excess in infected blood or whether we have at the same time a corresponding alteration in the iso- and hetero-agglutinin contents of the serum.

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* "On the Presence of Hæmagglutinins, etc., in the Blood obtained from Infectious and Non-infectious Diseases in Man," 'Roy. Soc. Proc.,' 1909, B, vol. 80, p. 531.

Mention has already been made of the fact that in every case where the serum of an infected animal was found to possess the property of clumping its own erythrocytes to a considerable degree, it also agglutinated markedly those of other members of the same species.

It was further observed that the plasma of infected animals frequently appeared to agglutinate the red cells of animals belonging to different species to a greater extent than normal. The case of human trypanosomiasis already referred to presented an excellent example of an increased capacity on the part of an infected serum to agglutinate foreign erythrocytes. The serum of this case clumped the red corpuscles of rats, guinea-pigs and rabbits in a remarkable manner. A few drops of the inactivated serum when added to an equal volume of the blood of one of these animals caused intense agglutination in a few seconds at room temperature. The action of normal human sera on these corpuscles was much slower and did not approach that of the former in intensity.

Experiments were undertaken with the object of comparing quantitatively the auto-, iso-, and hetero-agglutinin in the blood of several infected animals with that existing in the blood of normal animals of the same kind.

Technique.—The method adopted was that previously used for comparing the amount of auto- and iso-agglutinin in the defibrinated plasma separated from the red cells at 37° C. with that present in the serum obtained from blood which had clotted at 0° C.

Inactivated defibrinated plasma was prepared from normal Rabbit 1 and from Rabbit 896 (infected with *T. dimorphon*), and a 5 per cent. suspension of washed erythrocytes from the following animals: normal Rabbits 1 and 2, Rabbit 896 and a normal horse and guinea-pig.

From the results of this experiment, details of which are given in Table IV, and from other observations of the same kind one is led to conclude that in the blood of infected animals, in addition to an excess of auto-agglutinin, there is also frequently a corresponding increase in iso- and hetero-agglutinin.

The question now arises as to whether these reactions are manifestations of the same body or of different specific agglutinins. The procedure usually adopted for the solution of problems of this nature is the saturation of a portion of the serum by the red cells of one of the varieties in

Table IV.—Comparison of the Amount of Auto-, Iso-, and Hetero-agglutinin in the Plasma of Rabbit 896 (infected with *T. dimorphon*) and Normal Rabbit 1.

Equal volumes of red cell suspension and diluted plasma used. Temperature of experiment 0° C.		Result.
5 per cent. suspension of washed erythrocytes in normal saline solution.	Plasma of Rabbits 1 and 896 diluted with increasing amounts of normal saline solution.	
Rabbit 1	Rabbit 1 undiluted	Complete agglutination in 20 mins.
” 1	” 896 ”	” ” 20 ”
” 2	” 1 ”	” ” 20 ”
” 2	” 896 ”	” ” 20 ”
” 896	” 1 ”	” ” 20 ”
” 896	” 896 ”	” ” 20 ”
Horse	” 1 ”	” ” 10 ”
	” 896 ”	” ” 10 ”
Guinea-pig	” 1 ”	Marked ” 45 ”
	” 896 ”	Complete ” 45 ”
Rabbit 1	” 1 diluted with twice its vol. of 0·9 p. c. NaCl solution	Nil ” 45 ”
” 1	” 896 ”	Complete ” 20 ”
” 2	” 1 ”	Slight ” 20 ”
” 2	” 896 ”	Complete ” 20 ”
” 896	” 1 ”	Nil ” 20 ”
” 896	” 896 ”	Complete ” 20 ”
Horse	” 1 ”	Marked ” 45 ”
	” 896 ”	Complete ” 15 ”
Guinea-pig	” 1 ”	Nil ” 45 ”
	” 896 ”	Complete ” 15 ”
Rabbit 1	” 1 diluted with 4 times its vol. of 0·9 p. c. NaCl solution	Nil ” 15 ”
” 1	” 896 ”	Complete ” 15 ”
” 2	” 1 ”	Nil ” 15 ”
” 2	” 896 ”	Complete ” 30 ”
” 896	” 1 ”	Nil ” 30 ”
” 896	” 896 ”	Complete ” 30 ”
Horse	” 1 ”	Slight ” 30 ”
	” 896 ”	Complete ” 30 ”
Guinea-pig	” 1 ”	Nil ” 30 ”
	” 896 ”	Complete ” 20 ”
Rabbit 1	” 896 diluted with 6 times its vol. of 0·9 p. c. NaCl solution	” ” 20 ”
” 2	” 896 ”	” ” 30 ”
” 896	” 896 ”	” ” 30 ”
Horse	” 896 ”	Marked ” 45 ”
Guinea-pig	” 896 ”	Complete ” 15 ”
Rabbit 1	” 896 diluted with 9 times its vol. of 0·9 p. c. NaCl solution	” ” 20 ”
” 2	” 896 ”	Partial ” 45 ”
” 896	” 896 ”	Complete ” 30 ”
Horse	” 896 ”	Trace ” 45 ”
Guinea-pig	” 896 ”	Complete ” 15 ”
Rabbit 1	” 896 diluted with 14 times its vol. of 0·9 p. c. NaCl solution	Slight ” 45 ”
” 2	” 896 ”	Nil ” 45 ”
” 896	” 896 ”	Slight ” 45 ”
Horse	” 896 ”	Nil ” 45 ”
Guinea-pig	” 896 ”	Slight ” 45 ”

question, and then, after allowing reaction to take place for some hours, centrifugalising and examining the extracted serum regarding its agglutinating action on the red cells of the kind used for extraction and also on the other varieties of erythrocytes.

Malkoff,* adopting this technique, arrived at the conclusion that there exist in goat's serum, which is capable of agglutinating the erythrocytes of many kinds of animals, different specific agglutinins, each of which has a specific affinity for the corresponding variety of red cells.

Experiment.—(1) One volume of defibrinated plasma of Rabbit 896 (infected with *T. dimorphon*) was extracted for 12 hours at 0° C. with an equal volume of the undiluted red blood cells of the same animal. (Extracted plasma A.) (2) Here the proportion of plasma to erythrocytes was one to five. (Extracted plasma B.) (3) One volume of the same plasma was treated with one volume of normal horse's red cells. (Extracted plasma C.)

A 5-per-cent. suspension of washed erythrocytes was prepared from the following animals: Rabbit 896, normal Rabbit 1, normal Donkeys 1 and 2, a normal guinea-pig, horse, *Macacus rhesus*, *Cercopithecus callitrichus*, and human being.

The results indicate that complete extraction of the infected plasma of Rabbit 896 by its own erythrocytes and those of a normal horse does not completely destroy the agglutinating action of the plasma on the red blood cells of other animals, although it is to be noted that in most cases when the plasma had been extracted with five times its volume of its own red cells there was a marked lessening or even total disappearance of this action. This diminution of the agglutinating action of the plasma cannot be explained by mere dilution with the small amount of saline solution adhering to the red cells, as the plasma still caused marked agglutination after the addition of fifteen times its volume of 0·9 per cent. NaCl solution.

It is doubtful, however, whether experiments of this kind really have the importance that has been assigned to them by Malkoff and others.

Landsteiner and Sturli† using normal horse and dog serum and 11 varieties of erythrocytes, confirmed Malkoff's observation that saturation of the serum with one kind of red blood cell deprived it of the power to agglutinate this variety, and this only. They furthermore showed that red cells which had already been once completely agglutinated were still able to react with another kind of serum, and that the new serum after the reaction had lost its power to agglutinate fresh corpuscles of the same kind. Hence, as

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* "Beiträge zur Frage der Agglutination von rother Blutkörperchen," 'Deutsche Med. Woch.,' 1900, No. 14.

† "Ueber die Hämagglutinine normaler Sera," 'Wien. Klin. Woch.,' 1902, p. 38.

Table V.—Agglutinating Action of Plasma which had been previously Extracted with Red Blood Cells.

Equal volumes of red cell suspension and plasma used. Temp. of experiment 0° C.		Result.
5 per cent. suspension of washed erythrocytes in normal saline solution.	Untreated plasma of Rabbit 896 (infected with *T. dimorphon*) and also plasma of the same animal extracted with red cells as follows: A, with an equal volume of red blood cells of Rabbit 896. B, with five times its volume of red cells of Rabbit 896. C, with an equal volume of red cells of a normal horse.	
Rabbit 896	Untreated plasma	Complete agglutination in 10 mins.
	Extracted „ A	Partial „ 45 „
	„ „ B	No „ 45 „
	„ „ C	Complete „ 15 „
Rabbit 1	Untreated plasma	Complete „ 15 „
	Extracted „ A	„ „ 30 „
	„ „ B	No „ 45 „
	„ „ C	Complete „ 15 „
Donkey 1	Untreated plasma	Complete „ 15 „
	Extracted „ A	Almost complete „ 45 „
	„ „ B	No „ 45 „
	„ „ C	Marked „ 45 „
Donkey 2	Untreated plasma	Complete „ 15 „
	Extracted „ A	„ „ 30 „
	„ „ B	Trace „ 45 „
	„ „ C	Complete „ 45 „
Guinea-pig	Untreated plasma	Complete „ 15 „
	Extracted „ A	„ „ 15 „
	„ „ B	Partial „ 45 „
	„ „ C	Complete „ 15 „
Horse	Untreated plasma	Complete „ 10 „
	Extracted „ A	„ „ 15 „
	„ „ B	Slight „ 45 „
	„ „ C	Partial „ 45 „
Macacus rhesus	Untreated plasma	Complete „ 15 „
	Extracted „ A	„ „ 30 „
	„ „ B	Slight „ 45 „
	„ „ C	Complete „ 30 „
Cercopithecus callitrichus	Untreated plasma	Complete „ 15 „
	Extracted „ A	„ „ 15 „
	„ „ B	„ „ 30 „
	„ „ C	„ „ 15 „
Human being	Untreated plasma	Complete „ 10 „
	Extracted „ A	„ „ 20 „
	„ „ B	Marked „ 45 „
	„ „ C	Complete „ 15 „

Landsteiner points out, the problem had assumed a very complex aspect, the enormous number of specific agglutinins in normal serum appearing uneconomic.

Landsteiner and Sturli suggest another hypothesis to explain these facts, namely, that during the process of agglutination some substance passes from the red cell to the serum, and that after complete agglutination the serum, in consequence of the combination, agglutinin + corpuscle substance, can no longer react with red cells of the same kind, but can with those of other animals. By this theory they maintain that the facts can be explained without the necessity for assuming the presence of an enormous number of differently acting substances or groups of substances in normal serum.

A certain amount of support is afforded this view by the observation of Landsteiner that a watery extract of the corpuscles of a turkey, when added to horse serum, almost completely prevents its agglutinating action on the red cells of the turkey, but only in a very slight degree on other kinds of blood. This last observation was subsequently confirmed by Lazar.* However, unless all traces of the stromata of the red cells had been removed from the hæmoglobin solution—and this is by no means an easy performance—an obvious explanation for this inhibiting action of such solutions would be that the stromata themselves had fixed the agglutinins present in the horse serum, and consequently there would be little, if any, left to act upon the red corpuscles. Naturally, in this case, the inhibiting action would be specific for the variety of red cells from which the hæmoglobin solution was made.

The fact that the phenomenon of auto-agglutination is reversible allows one to approach the subject of specificity from a different point of view, namely, by extracting the completely agglutinated red cells with a small quantity of normal saline solution at 37° C., and then investigating the nature of the digest.

Experiment.—To 10 c.c. of defibrinated plasma of Rabbit 1035 (infected with *T. brucei*) were added 0·2 c.c. of the red cells of the same animal. After allowing the mixture to stand in the ice chest for 12 hours with occasional stirrings the supernatant plasma was decanted off, and the clumped red blood cells washed four times with at least 10 times their volume of normal saline solution at 0° C.; 0·2 c.c. of normal salt solution was then added to the agglutinated mass of red cells and the mixture allowed to digest at 40° C. for half an hour. At the end of this time no trace of agglutination was visible. The red cells were then quickly thrown down by centrifugalisation and the supernatant fluid removed (Digest solution).

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* "Ueber die Bedeutung der lipoiden Stoffe der rothen Blutkörperchen für den Mechanismus der Agglutination," 'Wien. Klin. Woch.,' 1905, p. 1012.

A 5-per-cent. suspension of red blood cells in normal saline solution was prepared from the following animals: Rabbit 1035 (infected with *T. brucei*), normal Rabbit A, Donkey 2 (infected with *T. rhodesiense*), normal Donkey A, Goat 1041 (infected with *T. rhodesiense*), normal Goat A, and from a normal rat, guinea-pig, dog, horse, *Macacus rhesus*, *Cercopithecus callitrichus*, and human being.

Table VI.—Agglutinating Action of a Solution Obtained by Digesting Auto-agglutinated Red Blood Cells with Normal Saline Solution at 40° C.

Equal volumes of red cell suspension and defibrinated plasma or digest solution used. Temperature of experiment 0° C.		Result.
5 per cent. suspension of washed erythrocytes in normal saline solution.	Untreated defibrinated plasma of Rabbit 1035 (infected with nagana), and solution obtained by digesting the auto-agglutinated red cells of the same animal with normal saline solution at 37° C.	
Rabbit 1035 (infected with *T. brucei*)	Defibrinated plasma	Complete agglutination in 10 mins.
	Digest solution	” ” 10 ”
Normal Rabbit A	Defibrinated plasma	” ” 10 ”
	Digest solution	” ” 10 ”
Donkey 2 (infected with *T. rhodesiense*)	Defibrinated plasma	” ” 10 ”
	Digest solution	” ” 30 ”
Normal Donkey A	Defibrinated plasma	” ” 15 ”
	Digest solution	Slight ” 45 ”
Goat 1041 (infected with *T. rhodesiense*)	Defibrinated plasma	Complete ” 10 ”
	Digest solution	No ” 45 ”
Normal Goat A	Defibrinated plasma	Complete ” 10 ”
	Digest solution	No ” 45 ”
Rat	Defibrinated plasma	Complete ” 15 ”
	Digest solution	” ” 20 ”
Guinea-pig	Defibrinated plasma	” ” 15 ”
	Digest solution	” ” 20 ”
Dog	Defibrinated plasma	” ” 5 ”
	Digest solution	” ” 10 ”
Horse	Defibrinated plasma	” ” 10 ”
	Digest solution	Partial ” 45 ”
Macacus rhesus	Defibrinated plasma	Complete ” 15 ”
	Digest solution	” ” 30 ”
Cercopithecus callitrichus	Defibrinated plasma	” ” 15 ”
	Digest solution	” ” 15 ”
Human being	Defibrinated plasma	” ” 10 ”
	Digest solution	” ” 15 ”

N.B.—The four specimens of normal saline solution which had been used for washing the clumped red cells were also examined. With the exception of occasional traces in the first, no agglutinin was found in these solutions.

The capacity of the untreated plasma of Rabbit 1035 and of the solution prepared by digesting the agglutinated red cells with normal saline at 0° C. to agglutinate these different erythrocytes was then examined.

The information obtained from observations of this kind is extremely interesting. In the experiment recorded a substance was extracted from the auto-agglutinated erythrocytes of a rabbit infected with *T. brucei* which clumped not only its own erythrocytes and those of other rabbits, but also the red cells of many other animals of different species. In other words it would appear that the auto-agglutinin is not a body equipped with a high degree of specificity, but that it can also act as iso- and hetero-agglutinin on the erythrocytes of other rabbits and those of animals of widely different species.

Value of the Phenomenon as a Diagnostic Sign.—Before discussing this question it is necessary to emphasise the importance of careful observation in determining whether a certain blood really agglutinates or not. So far as can be gathered from the papers in which the existence of the phenomenon in trypanosomiasis has been recorded, it has been invariably decided from the examination of cover-slip preparations of the blood. Although a considerable degree of auto-agglutination is easily recognised in a well-made cover-slip preparation, yet it is often extremely difficult, or even impossible, to decide whether the red cells are really agglutinated when the phenomenon is not so distinct. A certain amount of massing together of the erythrocytes is frequently evident at the edges of even the best cover-slip preparations of normal blood, whereas if the slide and cover-slip be not perfectly clean the red cells are found to be anything but evenly distributed, but are grouped together into little masses and rouleaux, separated from one another by plasma—an appearance closely resembling that to be observed in infected blood when the amount of auto-agglutination is slight. On the other hand, a slight degree of auto-agglutination can be easily obscured by pressure on the cover-slip resulting in the separation of the erythrocytes one from the other.

Furthermore, it has been shown that small amounts of auto-agglutinin exist constantly in the blood of many normal animals. In horses and donkeys auto-agglutinin is sometimes present in such an extent as to give rise to a more or less characteristic appearance in cover-slip preparations. This is specially the case when the preparations are made out of doors at a somewhat low temperature. However, I have never observed in cover-slip preparations of the blood of normal animals a condition approaching in intensity the well-marked clumping obtaining in infected cases. When a high degree of auto-agglutination exists the corpuscles are seen to have become agglomerated into tight clumps, the outlines of the individual cells being indistinct, or even completely lost, so that the clumps appear to consist of red cells which have fused together into a homogeneous mass. In order to evoke as characteristic an appearance as possible the preparation should be made at the lowest temperature practicable.

The next point to be considered is whether auto-agglutination is a constant feature in trypanosomal infections.

Martin, Lebœuf, and Roubaud* stated that in the large number of cases of human trypanosomiasis examined by them in the French Congo, auto-agglutination was always present. In the tables appearing in their report, the condition of the blood as regards auto-agglutination is indicated by numbers from 0 to 10, the cipher meaning that there is no agglutination, whereas the greatest degree of agglutination is indicated as 10; the intermediate figures denote intermediate degrees of agglutination.

In view of the technique used by them—the mere examinations of cover-slip preparations of the fresh blood—such a classification appears to be a somewhat unwarrantable refinement.

Todd† in a recent paper classifies as regards auto-agglutination a large number (1406) of cases examined by Dutton and himself in the Congo Free State. Of the 395 cases in which auto-agglutination was present, trypanosomes were found in only 183. However, as Todd himself states, probably because of the insufficient search for them (the cases were seen and examined on one occasion only), trypanosomes were present much more often than they were found.

Later in the same paper it is stated that only in three cases were trypanosomes not present when an extremely well marked auto-agglutination was recorded. One of these was a case of relapsing fever; another was a much emaciated marasmic individual, and the third was a case of syphilis.

Regarding the frequency of the phenomenon in the blood of experimentally infected animals, it need only be stated that as a rule auto-agglutination is best marked in the blood of the larger animals, *e.g.* horse and donkey. It is usually also very distinct in the monkey, dog, rabbit, and goat. In the rat, mouse, and guinea-pig it is generally slight or absent.

In the ordinary course of events the infected animal shows parasites in its blood for some time before a distinct auto-agglutination develops. Occasionally, however, auto-agglutination appears before trypanosomes have been found in the blood. I have had under observation a goat, infected with *T. gambiense*, in which trypanosomes have never been seen in the blood, but in which a well marked auto-agglutination had developed. The blood was shown to be infective by injection into rats.

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* 'Rapport de la Mission d'Études de la Maladie du Sommeil au Congo Française,' 1906—8, p. 281.

† "A Note on the Occurrence of Auto-agglutination of the Red Cells in Human Trypanosomiasis," 'Bull. Soc. Path. Exot.,' 1910, p. 438.

The last point to be decided in considering the value of the phenomenon as a diagnostic sign is whether it occurs in other diseases besides trypanosomiasis. In addition to the three cases mentioned by Todd, auto-agglutination has occasionally been noted in persons suffering from other diseases than Sleeping Sickness.

Klein,* in 1890, found auto-agglutination of the red blood cells in a case of hepatic cirrhosis (Hanot). Dudgeon† mentions the case of a West Indian negro which was considered to be one of tertiary hepatic syphilis, where the blood exhibited spontaneous clumping. He also found auto-agglutinin in the blood of a case of long standing epilepsy. Martin and Darré‡ assert that the phenomenon is to be met with in certain forms of icterus due to hæmolysis.

Quite recently Nattan-Larrier§ described the existence of auto-agglutination in rats infected with the *Spirilla obermierei.* It is interesting to note in this connection that of Todd's three cases which exhibited well marked auto-agglutination, but were not infected with trypanosomes, one was a case of relapsing fever and another a case of syphilis, as was also the only definite instance of the phenomenon seen by Dudgeon. It is of importance to know whether auto-agglutination is often present in spirochaetal infections.

In conclusion it may be stated that in the light of the information obtainable a well-marked degree of auto-agglutination of the red blood cells is an extremely rare occurrence, apart from infection with trypanosomes.

Summary.

Auto- and iso-agglutinin are present in the blood of cases of Sleeping Sickness and of animals infected with trypanosomiasis.

Reaction between auto-agglutinin and red blood cells takes place only at low temperatures.

Auto-agglutinin can be removed from plasma by absorption with the erythrocytes of the same animal at 0° C.

The reaction between auto-agglutinin and red blood cells is reversible.

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* "Ueber die Untersuchung der Formelemente des Blutes und ihre Bedeutung für die praktische Medicin," 'Wien. Klin. Woch.,' 1890, Nos. 36—40.

† *Loc. cit.*

‡ 'Bull. et Mémoires Soc. Méd. des Hôpitaux de Paris,' 1909, p. 599.

§ "L'Autoagglutination des Hématies dans la Spirillose Expérimentale," 'Bull. Soc. Path. Exot.,' 1910, p. 425.

Auto-agglutinin exists in small amounts in the blood of many normal animals.

Auto-, iso- and hetero-agglutinin are frequently present in much greater amount in the blood of infected animals than in that of normal animals, and it is due to this fact that clumping of the red blood cells is often visible in fresh cover-slip preparations of the blood of infected animals.

From the red blood cells of an infected animal which have been agglutinated in the cold by the plasma of the same animal an active substance can be extracted with normal saline solution at 37° C.

This substance agglutinates not only the red cells of the same animal and other members of the same species, but also those of many animals of different species.

It is to be inferred from the information at present available that a marked degree of auto-agglutination of the red blood cells is an extremely rare occurrence apart from an infection with trypanosomes.

Address of the President, Sir Archibald Geikie, K.C.B., at the Anniversary Meeting on November 30, 1910.

The year which has passed since our last Anniversary will be memorable in our annals for the losses which death has brought upon the Royal Society. First and most conspicuous is the decease of our Patron, His revered Majesty, King Edward VII. During his brief but eminently beneficent reign, His Majesty continued to manifest the same interest in the advancement of science which he had always shown before his accession to the Throne. We mourn that a life so devoted to the cause of peace and progress should have been cut off so soon. King George V has honoured us by stepping at once into his father's place as Patron of the Royal Society, and has subscribed his name in our Charter-book. His Majesty as Prince of Wales found many occasions to show his appreciation of science, and his interest in its progress. We feel confident that under his enlightened rule the advancement of Natural Knowledge will continue to receive his support and encouragement.

The deceased Fellows are:

Dr. Ludwig Mond, died December 11, 1909.
Dr. Shelford Bidwell, died December 18, 1909.
Sir Charles Todd, died January 28, 1910.
Edward Saunders, died February 6, 1910.
Sir Robert Giffen, died April 12, 1910.
Sir William Huggins, died May 12, 1910.
John B. N. Hennessey, died May 23, 1910.
C. Greville Williams, died June 15, 1910.
The Rev. Robert Harley, died July 26, 1910.
Dr. Sydney Ringer, died October 14, 1910.

The deceased Foreign Members are:

Prof. Friedrich Kohlrausch, died January 17, 1910.
Prof. Eduard F. W. Pflüger, died March 17, 1910.
Prof. Alexander Agassiz, died March 28, 1910.
Prof. Stanislao Cannizzaro, died May 10, 1910.
Prof. Robert Koch, died May 27, 1910.
Prof. Giovanni Schiaparelli, died July 4, 1910.
Dr. Melchior Treub, died October 3, 1910.

On the list of Foreign Members of the Society we have thus to mourn the loss of no fewer than seven illustrious names. Of these there is none which we more deeply regret than that of ALEXANDER AGASSIZ. His frequent visits to Europe brought him into closer personal contact with the Fellows of the Royal Society than is usually possible for our Foreign Members. Only last summer he came once more among us with apparently little diminution of his characteristic capacity for work. But it was his last public appearance, for he died a week or two afterwards on his homeward passage across the Atlantic.

His genius for original research, the unwearied activity with which he pursued those lines of enquiry to which he specially devoted himself, and the generous prodigality with which he placed his ample fortune at the service of science in the investigations which he led and inspired, placed him at the head of the oceanographers of his day. Year after year he traversed, dredged, and sounded the ocean through many different latitudes, bringing back from each cruise enormous collections of material, which went to increase the treasures of that Museum of Comparative Zoology which his father had planned at Harvard, and to which he himself had devoted the strenuous energies of his life. Besides adding much to our knowledge of the fauna of the deep, he ever had an eye for the great physiographic problems which the oceans, their coasts, and their islands present. The pile of goodly volumes in which his incessant labours are chronicled form one of the most remarkable monuments which have been reared in our day by the genius, enterprise, and enthusiasm of a single man.

By the death of STANISLAO CANNIZZARO, at the ripe age of 84, Italy has been deprived of her foremost chemist, and science has to regret the loss of one of her illustrious students, who by his generalisations on chemical combination did so much to place modern chemistry on a sound basis. It happened that at the time of his death the senior Secretary of the Royal Society and myself were in Rome as delegates of the Society to the meeting of the International Association of Academies. We were glad to avail ourselves of the opportunity of attending the funeral of our deceased Foreign Member. It was a touching sight to find that in the class-room where he had so long taught and where his diagrams were still hanging on the walls, the coffin had been placed on his lecture-table and was guarded by a body of his students, who bore it thence on their shoulders, through a dense crowd of mourners to the hearse. Representatives of science from far and near followed the body to the grave.

A veteran astronomer has passed away in GIOVANNI SCHIAPARELLI. From the time of his discovery of the planet Hesperia, made when he was only six and twenty years of age, he prosecuted an active and successful study of the heavens, extending over some forty years at the observatory of Milan.

His notable identification of the orbits of meteors with those of comets, his minute delineation of the surface of the planet Mars, and his subsequent studies of Mercury and Venus, have made his name a household word in astronomical science.

In ROBERT KOCH we have to deplore the loss of one who conferred inestimable benefits on the science of bacteriology. To him we owe the isolation of the tubercle bacillus, the proof that it is the cause of tuberculosis, and the product, tuberculin, for the treatment of this calamitous disease. He has made modern bacteriology possible by his elaboration of methods for the culture of bacilli.

FRIEDRICH WILHELM KOHLRAUSCH, who died in January last, in his seventieth year, will be remembered for his investigation of the methods of measuring magnetic and electrical quantities, for his laborious researches into the conducting power of electrolytic solutions, which formed the foundation of the modern electrolytic theory of solution, and for the great service which he rendered by insisting on the necessity of practical instruction in the laboratory for the teaching of physics.

In MELCHIOR TREUB botany has lost an esteemed and eminent worker. A master of technique, with high intellectual gifts, he attacked many important problems and materially advanced knowledge in the diverse domains of plant physiology, cytology, morphology and geography, presenting his results with great lucidity and grace of style. His scientific work was mainly done while he was engrossed in the official duties of Director of the Buitenzorg Botanic Garden in Java: duties performed for nearly thirty years with such success that the practical benefits which resulted from them to pharmacology, forestry and tropical agriculture are comparable with his scientific contributions. His sympathies were as wide as his interests; and his memory will live in the work which he helped others to achieve as well as in his own.

Coming now to the losses from our Home List during the year that has passed, we have to mourn the death of one of the great historical figures of the Royal Society—SIR WILLIAM HUGGINS. It would be out of place in this brief address to attempt even a summary of the achievements of his distinguished career, but on this anniversary occasion our thoughts naturally turn to the recollection of the salient features of that career which shed such lustre on the Society. Soon after the invention of spectrum analysis as a practical method of investigation was accomplished, only fifty years ago, by Kirchhoff and Bunsen, Huggins resolved to devote himself to the application of this new method to astronomical problems, and, as we all know, he thereby laid the foundations of the wonderful science of astrophysics. His early observations of the spectra of the stars, made before any of the apparatus now so appropriate for such purposes had been evolved or even thought of, showed his close application and the exquisite refinement of his appliances. In rapid succession he informed us of the similarities in constitution between some types among the fixed stars and our own Sun, and of the marked differences shown by other types, thus leading on to the great fundamental subject of the classification of the stars and initiating the discussion of the order of their evolution. The early application of photography to the spectra of objects whose light is so feeble demanded wonderful patience and skill: it was rewarded with an immediate crowd of physical results in the registration of spectra far beyond the visible limit in the violet, among which the recognition of the second spectrum of hydrogen may be recalled. So, too, at a later time, when the four lines of the ordinary spectrum of this gas were shown by Balmer to belong to a very definite algebraic series, which ought to include numbers of other lines whereof laboratory experiments showed no trace, it was Huggins who called in the aid of the celestial laboratory of the stars and pointed to the exact succession of the missing lines in the spectra of certain stars—thereby infusing new zeal into the efforts of theorists to unravel the secret of the origin of the spectrum.

When the same refined appliances were turned to the scrutiny of the nebulæ, which are spread over such vast regions of the sky, the news soon came that the problem raised by William Herschel and his successors had now been definitely solved by the discovery that these nebulæ were not all clusters of stars, but that some of them shone as masses of glowing gas. In solar work reference may be made to Huggins' proposal to render the flame-prominences visible in open day by great optical dispersion, and also, though the result was less satisfactory, to his persistent attempts to photograph the solar corona by aid of coloured screens. But perhaps the most brilliant of his achievements was his early conception, undeterred by full knowledge of the difficulties which had to be faced, that it might be possible to measure the velocities of approach or recession of the stars by spectroscopic means. In a masterly investigation he proved to the world that his expectation was well grounded, and that the development of instruments specially adapted for this new outlook into astronomy demanded vigorous prosecution. His other laboratory work, all designed for the elucidation of astronomical problems, can only be referred to, such, for instance, as his research into the nature of the luminosity of radium salts.

Sir William Huggins was elected into the Royal Society as far back as 1865. During his long association with us he took the keenest interest in the affairs and the success of the Society. He was repeatedly elected into the Council, where his total length of service amounted to no less than sixteen years. He served as Vice-President for four years, and we count it as one of the distinctions of the Society in our time that he held the office of our President for five years. Thus, not merely for the lustre reflected from his high reputation in science, but for the personal service which he so willingly and effectively rendered in the conduct of its business, the Royal Society gratefully and affectionately cherishes his memory.

But, great as was his position in scientific discovery, it is within the personal knowledge of most of us that in character he was equally great. His was an ideal life, dedicated throughout to the sublime science for which he did so much, and during many years happy in the devoted companionship and co-operation that eased, and at the same time stimulated, the arduous work of a solitary astronomer.

DR. LUDWIG MOND will be remembered not only for his eminence in chemistry, which was recognised by his election into this Society, but for the indomitable courage and sagacity with which he brought his discoveries into successful practical operation on a commercial scale, and not less for the well-considered and large-hearted liberality wherewith he dispensed the wealth which rewarded his success. The Royal Society has good cause to cherish his memory as that of a genial Member who took an active interest in its affairs, affording it at all times the benefit of his business experience, and ever ready to aid financially any of its enterprises which seemed to him to stand in need of assistance. By his will also he has left a munificent benefaction whereby the Society will ultimately be enriched.

The other deceased Fellows on the Home List are Dr. Shelford Bidwell, Sir Robert Giffen, the Rev. Robert Harley, Mr. John B. N. Hennessey, Dr. Sydney Ringer, Mr. Edward Saunders, Sir Charles Todd, and Mr. C. Greville Williams.

The Report of the Council now presented to the Fellows contains a record of the main features of the work of the Society for the past year. To one or two parts of this work I wish to make brief allusion. It will be seen that, acting on the recommendation of the President and Council of the Society, the Government of this country has agreed to continue its subscription to the International Association of Seismology for six years more, up to the end of March, 1916. This prolongation of the adhesion of Great Britain is eminently desirable, in order that time may be allowed for the consideration of the best means of securing effective international co-operation in seismological observation and enquiry. In this branch of science our country has a special interest, for it was here that modern observational seismology was begun many years ago, and that the first network of observing and registering seismological stations was established over the face of the globe. To the wide experience, great practical skill, and unwearied enthusiasm of our colleague, Dr. John Milne, the establishment and maintenance of that network of stations have been entirely due. With but little financial assistance from outside, he has borne the whole burden of the organisation, as well as of the voluminous correspondence which it entails with all parts of the world. The valuable service which he has thus rendered to the study of earthquakes has been universally recognised, and there is a widespread conviction that the system of observing stations which he has created is worthy of being made a national undertaking. The whole question of the future of seismology in this country must soon be seriously considered. Meanwhile, I would express my own personal hope that means will be found to place on a more permanent footing the work which Dr. Milne has originated and conducted, and to carry it on as successfully as in the past, but with an enlarged staff and more generous financial aid.

The Fellows are aware that for many years past the Society, at the request of different departments of Government, has undertaken the investigation of various diseases with the view of ascertaining their cause, and, if possible, of suggesting methods of treatment and cure. Chief among these enquiries is that of the appalling disease Sleeping Sickness. From the Report of the Council it will be seen that, although much important information has been obtained in Uganda, the investigation has had to be extended beyond the limits that originally seemed to be requisite, and that probably much still remains to be done before the conditions can be definitely stated in which trypanosome diseases are spread in tropical Africa. The work of those enquirers who are busy in London endeavouring to discover an effective drug for the treatment of trypanosomiasis is still in progress, with results which are so far encouraging for further investigation.

One of the most important statements in the Council's Report is that which has reference to the Gassiot Committee and the future of Kew Observatory. The arrangement therein detailed was the subject of long and careful enquiry and discussion. The Gassiot Committee has now been reconstituted and enlarged so as to make it an effective scientific body of advice in regard to magnetic, seismological or other geophysical observations which are to be conducted under the direction of the Meteorological Office.

Fellows of the Royal Society should be aware that they are divisible into two classes, those who were elected before 1878 and those who were elected after that year. The distinction is a pecuniary one. The older group paid £10 of entrance fee and an annual subscription of £4, which they still continue to pay. They are a dwindling band which now numbers only 57.

The second and younger group, by the institution of what is called the Fee-Reduction Fund, are relieved of the payment of an entrance fee and their annual contribution is reduced to £3, the remaining £1 being paid out of that Fund. When this arrangement was made it seems to have been calculated that the original capital sum of £10,111 5*s.* (which was raised by voluntary contributions), with the invested interest accruing from it, would yield an annual income of £600, which was estimated to be sufficient to meet the highest demand that was likely to be made on the Fund. But these calculations have proved erroneous, partly no doubt on account of the fall in the rate of interest and partly also because younger men have been elected into the Society than was formerly the case, so that the increase in the participators in the benefit of the Fund has not been balanced by deaths to the extent anticipated.

Consequently now, thirty years after the foundation of the Fund, the income, instead of amounting to £600 per annum, has only reached £467 4*s.* 9*d.* while the payments this year should be £474, viz., £150 in respect of fifteen entrance fees and £324 towards the annual contributions of 324 Fellows elected since 1878 and still living. There is, thus, this year, for the first time a deficit, which amounts to £6 15*s.* The excess of the sum which should be paid beyond the income of the Fund will increase annually, though at a diminishing rate, and will probably ultimately amount to about £50. It is obvious, therefore, that some new arrangement will require to be made. The Fellows elected since 1878 might be called upon to increase their annual subscriptions, but such a call would probably be felt to be both inconvenient and undesirable. An alternative would be to increase the capital of the Fund, and this would undoubtedly be the more acceptable solution of the difficulty. If the Treasurer could be put in possession of a sum of at least £1,000 he would be placed in a satisfactory position in regard to this portion of the Society's finances. So long as the deficit remains small the excess of income could be devoted to the increase of the capital, and consequently the sooner the sum required is obtained the better. I commend this matter to the consideration of the Fellows; among whom there are doubtless many who will be as glad to contribute substantial sums as were the original founders of the Fund.

MEDALLISTS, 1910.

COPLEY MEDAL.

The award of the Copley Medal has this year been made to one of our own countrymen, who has been more than fifty years a Fellow of the Royal

Society. Sir Francis Galton's life has been one of ceaseless activity in many varied departments of intellectual effort. Few of us can remember how he began as an enthusiastic explorer and geographer, "urged," as he confessed, "by an excessive fondness for a wild life," and with "the love of adventure" as his chief motive. He chose South Western Africa as the theatre of his exploration, penetrated into regions where no European foot had preceded him, and brought back with him a vivid impression of the scenery, physical geography, natural history, and ethnology of Damaraland and South Ovampoland. He embodied his observations in an interesting volume of travel published in 1853. That work showed that he was no mere hunter after game or seeker of adventure, but a shrewd and observant traveller, with his eyes open to every distinctive natural feature in the countries and their inhabitants. His experience in these African journeys led him to plan and to publish in 1854 his well-known and admirable hand-book, the "Art of Travel," which, as a pioneering treatise in the practical methods of scientific exploration, has proved of inestimable service to the travellers of the last half century.

Sir Francis at an early period of his career was led to interest himself in meteorology, which, as a science of observation, was then in its earliest infancy. With much labour and skill he constructed weather-charts, and discussed meteorological statistics. His zeal and success in these studies led to his being chosen a member of the Meteorological Council at its origin, and he remained in that position until the Council was superseded in 1901 by the Meteorological Office. He likewise acted as Chairman of the Royal Society's Committee of Management of Kew Observatory from 1888 till 1900, when the work of this Committee became merged in that of the National Physical Laboratory.

But it was not only in geography and meteorology that Sir Francis Galton manifested his versatile energies. He was much interested likewise in biological studies, especially in regard to questions of relationship and heredity. As far back as 1871 he began what has proved to be a voluminous and important series of contributions to these subjects. From his first paper, "Experiments in Pangenesis," down to his last volume on "Eugenics," his successive papers have shown a continuous development of ideas and conclusions. He was led from his early ethnological enquiries into the mental peculiarities of different races, to discuss the problems of Hereditary Genius, from the fundamental postulate that "a man's natural abilities are derived by inheritance under exactly the same limitations as are the form and physical features of the whole organic world." To obtain further data for the discussion of this subject, he carried out the elaborate statistical enquiries embodied in his "English Men of Science." Confident in the results of these researches, he proceeded after the manner of "the surveyor of a new country who endeavours to fix in the first instance, as truly as he can, the position of several cardinal points." His results in this quest were given in his "Inquiries into Human Faculty and its Development," published in 1883. A further contribution was made by him in 1889, when his work on "Natural Inheritance" appeared. His subsequent papers and essays on "Eugenics" have still further stimulated enquiry into a subject of such deep interest and transcendent importance in all efforts to improve the physical and mental condition of the human race.

It has seemed to the Council fitting that a man who has devoted his life with unwearied enthusiasm to the study and improvement of many departments of natural knowledge, whose career has been distinguished by the singleness and breadth of its aims, and by the generosity with which he has sought to further them, should receive from the Royal Society its highest award in the Copley Medal.

RUMFORD MEDAL.

The Rumford Medal has been awarded to Prof. Heinrich Rubens in recognition of the value of his researches in radiation. For many years he has been engaged in the experimental investigation of optical radiations of very long wave-length. In the course of this work he elaborated, in conjunction with Prof. E. F. Nichols, a method of isolating pencils of nearly homogeneous rays, using the fact that a non-metallic substance reflects very copiously waves of the same length as those to which it is opaque. If then a pencil of rays of mixed wave-lengths is reflected several times to and fro between mirrors of the same kind of substance, the rays finally emerging (the "Reststrahlen") have the wave-lengths of the kinds of light which the substance refuses to transmit. The light of other wave-lengths has been transmitted freely at each incidence, and by a sufficient number of reflections is ultimately removed from the pencil. By using different substances as reflectors, Prof. Rubens has isolated infra-red light of various wave-lengths up to as much as 96μ, or about 0·1 of a millimetre; while, on the other hand, purely electric waves have been produced of wave-lengths as small as 2 millimetres. He has thus enormously extended our knowledge of the infra-red spectrum. Moreover, in conjunction with colleagues, he has investigated the absorbing and reflecting powers of substances for these long wave-length rays. He has shown that, for radiation of wave-length even less than ten times the wave-lengths in the visible spectrum, the reflecting and absorbing powers of metals and alloys are determined by their electric conductivities alone, in accordance with Maxwell's theory. It followed from Maxwell's own observations on the absorption of gold-leaf for visible light that agencies more complex than conductivity must be involved for these shorter wave-lengths.

Prof. Rubens has recently applied to the measurement of the long infrared wave-lengths a quartz interferometer, and among other results, he has found that the refractive index of water, for waves of length about 82μ, is of the same order as for waves in the visible spectrum, while for the shortest Hertzian waves yet examined, about 2000μ, it is as high as 9.

These examples will serve to illustrate how much Prof. Rubens has already done to bridge the gap between optical radiations and electric waves produced by direct electric agency, and how much more is to be expected from him in the investigation of the interval still remaining in which such fundamental changes of properties take place.

ROYAL MEDALS.

The awards of the two Royal Medals given annually by our Patron the King have received His Majesty's approval.

One of these Medals has been assigned to Prof. Frederick Orpen Bower in recognition of the great merit of his contributions to morphological botany, of which department of science he is the acknowledged leader in Great Britain. Prof. Bower's early studies in this field (1880—82), on the genera *Welwitschia* and *Gnetum*, were marked by the discovery of the true nature of the two persistent leaves in *Welwitschia*. The next period of his work was given to a study of the morphology of the leaf. He developed in 1884 the idea of the phyllopodium or leaf-axis, and discussed in 1885 the apex of the leaf in *Osmunda* and *Todea*. This latter study was cognate to subsequent researches, the results of which were given in 1886 in a review of "Apospory and Allied Phenomena." This work, of much intrinsic interest, is important as having led its author to formulate the views advanced in 1890 in a memoir on "Antithetic as distinguished from Homologous Alternation [of Generation] in Plants." Another memoir, published in 1889, on "The Comparative Examination of the Meristems of Ferns as a Phylogenetic Study," prepared in the light of the then received belief that the leptosporangiate ferns are the more primitive, was followed in 1891 by a discussion of this question in which Prof. Bower advanced morphological reasons for reversing the hitherto accepted phylogenetic order. The new conclusion has proved to be in accord with palæobotanical results, and marked another distinct step in the advancement of botanical science.

During the third period of his work, 1892—1903, Prof. Bower's papers, including an important series on the spore-producing members, have resourcefully maintained the antithetic doctrine, and have afforded a striking instance of the advantage of a well-considered working hypothesis as a guide to investigation. The career of morphological research here outlined has been recently crowned by the publication (1908) of a book on "The Origin of a Land Flora," which is one of the "most important contributions to the advancement of Natural Knowledge, published originally in His Majesty's dominions," within the period prescribed in respect of the award of Royal Medals.

The other Royal Medal has been adjudged to Prof. John Joly, who is eminent in two branches of science, geology and physics. This combination of studies has proved to be reciprocally fruitful to both departments. It was from his mineralogical interests that he was led to devise the steam calorimeter, which has enriched physics with an apparatus of high refinement. The use of this method was extended by him to the direct determination of the specific heats of gases at constant volume, a measurement dealing with minute quantities of heat under circumstances quite beyond the capabilities of the usual forms of calorimeter. Among many contributions to standard physical data, which are accepted and in use, may be instanced his determination of the density of saturation of steam. His meldometer, primarily intended for determining the melting points of mineralogical and geological specimens, has been the means of providing data for use in thermometry. He has devised and applied a method of determining the change of volume of rocks and other substances on fusion, which is a datum of primary importance for cosmical theories. He has carried out a refined research, with negative results, on the possibility of minute change of mass (as distinguished from weight) accompanying chemical combination. His recent extended investigations of the occurrence of radioactive substances in materials from various strata have been utilised for fundamental geological discussions. Of other useful inventions which he has introduced, one of the best known is the translucent block photometer.

Prof. Joly has made important contributions to the subject of colour photography, and devised some years ago a three-colour system in which all three colours are present on the same plate in the form of fine parallel lines or small dots.

He has also contributed substantially to the theory of biological processes, such as the ascent of sap in vegetation. Reference may likewise be made to his suggestive memoir on the Age of the Earth based upon a discussion of the chemical constitution of the Ocean.

DAVY MEDAL.

The Davy Medal has been assigned this year to Prof. Theodore W. Richards, as a mark of appreciation of the value of his work in the determination of the atomic weights of the elements. His researches on this subject have not been surpassed in comprehensiveness by those of any other chemist. He has himself determined the atomic weights of no less than 14 elements, and many other atomic weight determinations have been made under his direction and superintendence. The accuracy of the numbers obtained is certainly much higher than that which has been attained by any previous series of researches, and it is impossible to speak in too high terms of the ingenuity, the unremitting labour, and the masterly manipulation which Prof. Richards has brought to bear on his investigations.

In addition to this work on atomic weights, Prof. Richards has made many important contributions to physical chemistry, and it is probably no exaggeration to say that he has done more to raise the standard of accuracy in physico-chemical work than any other living chemist. Theoretical contributions to this branch of science are comprised in a series of papers on "The Possible Significance of Changing Atomic Volume," in which he suggests a relation between the energy of the atoms and their compressibilities. In order to test his hypothesis, he has made a long series of investigations on the compressibility of elements and compounds. He has determined this constant for nearly all the solid and liquid elements, and he has shown that the compressibility is a periodic function of the atomic weights. In electro-chemistry, Prof. Richards has made important determinations of the electro-chemical equivalent of silver, and he has supplied some of the most rigorous proofs of the universality of Faraday's Law.

DARWIN MEDAL.

To Mr. Roland Trimen, who was for many years Curator of the South African Museum, in Cape Town, the Darwin Medal has been awarded. His official position, and the duties it involved, enabled him to do admirable work in African zoology. His name will always stand with those of Bates and Wallace in the establishment and illustration of the theory of mimicry. In addition to his researches on that subject, he has done admirable systematic work, his descriptions of insects, especially the *Lepidoptera rhopalocera*, being models of accuracy and literary style. He, furthermore, rendered the greatest assistance to Charles Darwin, especially in his work on orchids—assistance the high value of which is acknowledged in a long series of that great naturalist's published letters.

SYLVESTER MEDAL.

The Medal which perpetuates the name and mathematical prowess of James Joseph Sylvester has this year been assigned to Dr. Henry Frederick Baker, in recognition of his work in the Theory of Functions, wherein he has shown himself to be a profound analyst. His book on the Abelian Functions, published in 1897, is a classic, and probably no better guide to the analytical development of pure mathematics has appeared during the last three-quarters of a century. While basing the argument of the work on the methods of Riemann, he never loses sight of the arithmetical ideas which we owe to Kronecker, Dedekind, and Weber, or of the geometrical notions brought to light by the labours of Clebsch, Gordan, Noether, and Klein. The critical insight which was thus in evidence marked him out a few years ago as the editor of Sylvester's Collected Papers. This work, which, with the approaching issue of the fourth and last volume, may be said to be complete, has been necessarily a difficult task, which besides making demands upon the resources of an accomplished mathematician has entailed no little editorial labour. Dr. Baker, by explanatory and critical observations, and by frequent ameliorations of the text, has done much to assist mathematical students. His scholarly work has resulted in a faithful record of the course of Sylvester's thought. It seems eminently fitting that the Sylvester Medal should be given to one who has erected so lasting a memorial to the great mathematician.

HUGHES MEDAL.

To Prof. John Ambrose Fleming the Hughes Medal has been awarded. For thirty years he has been actively engaged in researches in experimental physics, chiefly in the technical applications of electricity. He was an early investigator of the properties of the glow lamp, and elucidated the unilateral conductivity presented in its partial vacuum between glowing carbon and adjacent metal, a phenomenon which has been linked up recently with the important subject of the specific discharges of electrons by different materials. He has published in the scientific and technical press, and in technical text-books, many admirable experimental investigations and valuable expositions in the applications of electricity, as, for example, to electric transformers and wireless telegraphy. Of special interest and value for theory were the important results concerning the alterations in the physical properties of matter, such as the remarkable increase in the electric conductivity of metals, when subjected to very low temperatures, which flowed from his early collaboration with Sir James Dewar in investigating this domain. In recent years he has taken a prominent part in the scientific development of telegraphy by free electric waves.

The Action of Bacillus lactis aërogenes *on Glucose and Mannitol.* Part II.—*The Investigation of the* 2 : 3 *Butanediol and the Acetylmethylcarbinol Formed; the Effect of Free Oxygen on their Production; the Action of* B. lactis aërogenes *on Fructose.*

By G. S. WALPOLE, B.Sc. (Melbourne).

(Communicated by Dr. A. Harden, F.R.S. Received October 22, 1910,—Read January 19, 1911.)

(From the Bio-Chemical Laboratory, Lister Institute.)

A preliminary communication on some of this work has been published.* The present paper deals with the experimental part of the investigation in question, and some new results are recorded. On examining the action of *B. lactis aërogenes* on glucose, it was found that the carbon content of the products isolated was not so great as that of the original glucose employed. A further examination disclosed that a quantity of a neutral liquid was present containing the amount of carbon needed to explain this discrepancy. As, in the preliminary communication, justification was shown for calling this liquid "crude butylene glycol," the use of this convenient name will be continued here. The object of this investigation was to determine as far as possible the nature of the constituents of this material, and the proportions in which they are found therein.

The Conditions of Cultivation of the Organism.

In all the earlier experiments the organism (*B. lactis aërogenes*) was grown anaërobically in a medium containing 1 per cent. of Witte peptone, 2 per cent. of pure glucose, and an excess of chalk. Later, other sugars were used, and sometimes, when using glucose, the percentage of sugar employed was raised to 5, in order to obtain larger yields of one particular product per litre of medium treated. The flasks employed were of 1 litre capacity, and each was provided with a mercury trap which permitted the egress of the gases evolved. The fermentation was carried on under strictly anaërobic conditions, and the method of preparing the flasks was that employed by Harden.* While in the incubator they were agitated from time to time to distribute the chalk throughout the medium. The gas evolution was found to be vigorous during the first four days, but after two to four weeks ceased completely, and the quantity of unchanged sugar in the flask was very small indeed.

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* Harden and Walpole, 'Roy. Soc. Proc.,' B, 1906, vol. 77, pp. 399—405.

At first every flask removed was tested by plating on agar, but the practice was frequently omitted and, instead, a culture on sloped agar in a test-tube made and examined. The quantities of the various substances found are stated in the previous paper. In order to isolate the crude butylene glycol, the contents of the flask were first filtered from excess of chalk and then evaporated at 55° under diminished pressure to dryness, and the dry residue, consisting of calcium salts and peptone, extracted with alcohol. The alcohol extracts yielded on fractionation a colourless liquid, boiling at 181° to 183° (corr.) at 760 mm. pressure. The yield was very small, only amounting to about 1 gramme per litre of medium containing 20 grm. of glucose, but it was found possible to increase it by employing a medium containing 5 per cent. of glucose. In this way 8 grm. of the new substance containing 52·8 per cent. of carbon were obtained per litre of medium containing 50 grm. of sugar. This only accounts for about two-thirds of the missing carbon, and a rough estimate of the amount lost during the process of distillation and extraction was therefore made by dissolving 8 grm. of the material in 500 c.c. of a medium containing 5 grm. of Witte peptone, 6 grm. of calcium lactate, and 6·5 grm. of alcohol, and then extracting it in the manner described above. Only 5·2 grm. were recovered, the loss per 500 c.c. being therefore about 2·8 grm., and the loss per litre about 5·6 grm. This brings the total amount produced from 50 grm. of glucose to about 13·6 grm., slightly in excess of that required.

In one case a yield of 10·75 grm. was obtained from 1 litre of medium containing 5 per cent. of glucose. Generally, only 7 to 8 grm. of the "crude glycol" were obtained from each flask of 50 grm. of glucose, in spite of numerous attempts to improve the yield.

I. THE NATURE OF THE "CRUDE GLYCOL."

This substance boils at 181° to 183° (corr.) at ordinary pressure, and at 12 mm. the boiling point is from 83° to 84°·5 C.; it solidifies in the cold to a transparent mass, which melts indefinitely at about 28°, and shows to a marked degree the phenomena of super-cooling. It is slightly dextrorotatory, the value for $[\alpha]_D$ for different preparations varying from +0·46 to +0·71. A sample of crude glycol gave the value +1·6 for $[\alpha]_D$ when dissolved to the proportion 22 per cent. in a saturated aqueous solution of borax, while in aqueous solution it was +0·8. Dried over quicklime no decomposition apparently takes place at once, though, after several months, discoloration, diminution of viscosity, and development of a peculiar smell occur.

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* 'Jenner Inst. Trans.,' 1899, vol. 2, p. 126.

Samples of the natural product gave the following figures on burning:—

	C.	H.
	per cent.	per cent.
I	52·41	10·80
II	52·68	10·92
III	52·62	10·92
IV	52·71	10·93

The figures corresponding to the formula $C_4H_{10}O_2$ are 53·33 for C and 11·11 for H. It will be seen that the presence of a trace of moisture will account for this discrepancy, which will be referred to later.

Desiccation of the "Crude Glycol."

In order to determine the percentage composition of the desiccated natural "glycol" a sample several times redistilled *in vacuo* was distilled on to quicklime, sealed up for several weeks, and then distilled off again at 12 mm. pressure. No difference in boiling point was observed between the four consecutive fractions of several cubic centimetres, each of which was collected and at once hermetically sealed.

Melting-point.—These were now all frozen and very slowly warmed in a large bath from 5° to 30°, the operation taking two hours and the bath being vigorously stirred the while.

Sample I.—At 21° it became pasty and began to liquefy and did so more rapidly than IV. *Sample* II was not further examined. *Sample* III remained solid above 28°·5. *Sample* IV became pasty and began to liquefy at 21°, and completely liquefied at 28°.

It will be seen that the figures of Sample III, which had the highest melting point, correspond accurately to the formula $C_4H_{10}O_2$, but are considerably different from those of Nos. I and IV.

The value of $[\alpha]_D$ falls as the material distils.

Composition and Optical Activity of Desiccated "Crude Glycol."

No. of sample.	Wt. taken.	CO_2.	H_2O.	C.	H.	$[\alpha]_D$ in 35 per cent. aqueous solution.
				per cent.	per cent.	
I	0·1464	0·2836	0·1448	52·83	10·99	+1·41
III	0·2945	0·5770	0·2925	53·43	11·11	+1·21
IV	0·3190	0·6160	0·3164	52·66	11·09	
	0·3682	0·7147	0·3669	52·94	11·07	+0·92

Molecular Weight of the "Crude Glycol."—A molecular weight determination by Victor Meyer's method gave the value 80·6. The temperature employed was 215°, and 0·0435 grm. of glycol gave 12·6 c.c. of air at 15° and 776 mm. By Beckmann's freezing-point method the value 80 was obtained—

0·1386 grm. "glycol" in 22 c.c. aqueous solution gave $\Delta = 0·145$
0·2079 „ „ „ „ $\Delta = 0·307$

The formula weight of $C_4H_{10}O_2$ = 90.

Oxidation of the "Crude Glycol."

(1) *Oxidation in Aqueous Solution by Bromine in the Presence of Light. Formation of Diacetyl.*—To 0·9 grm. of crude glycol dissolved in 50 c.c. of water 3·25 grm. of bromine was added. The whole was well shaken from time to time and exposed to the light from an arc-lamp, care being taken not to allow the temperature of the mixture to rise to any considerable extent. After six hours a dilute aqueous solution of sulphur dioxide was added until the smell of bromine disappeared, and the mixture was saturated with sodium chloride. Distillation under a Young's still-head of 15 discs yielded some drops of a yellow liquid which possessed the characteristic smell of diacetyl and dissolved in excess of water.

Phenylosazone of Diacetyl.—This was prepared in order to confirm the identification of this product of the oxidation of "crude glycol." The solution of diacetyl obtained above was made up to 10 c.c. with water, 3 grm. of phenylhydrazine and 3 c.c. of 50 per cent. acetic acid added, and the whole kept at 100° for one hour. The phenylosazone deposited was recrystallised from alcohol and then melted at 241°·5 to 243°.

0·1147 grm. gave 20·6 c.c. N (moist) at 18°·5 and 765 mm.

Found N = 20·8 per cent. Calculated for $C_{16}H_{18}N_4$, N = 21·05 per cent.

The osotetrazone prepared from this by oxidation with potassium bichromate and acetic acid melted at 153°. The needles of this substance, recrystallised from acetone, were flexible, well defined, violet in colour, of great lustre, and melted at 155° to 156°, thus agreeing in every respect with the compound described by Pechmann.*

0·2613 grm. gave 47·25 c.c. N_2 (moist) at 17° and 763. 8 mm.

Found N = 21 per cent. Calculated for $C_{16}H_{16}N_4$, N = 21·4 per cent.

On reduction with phenylhydrazine the osazone melting at 245° is immediately re-obtained. All these reactions were also obtained with a few grammes of diacetyl prepared from methylacetoacetic ester.

Dioxime from Diacetyl.—Two grammes of crude glycol were converted into diacetyl by the method described and after reaction with hydroxylamine hydrochloride, 30 per cent. of the amount of oxime theoretically obtainable was isolated in the pure condition. This melted at 135°·5 to 136°·5, thus agreeing with the dioxime prepared from diacetyl obtained from methylacetoacetic ester. It gave the well-known dimethylglyoxime reaction with small quantities of nickel.† The yield of diacetyl by oxidation of the crude glycol, taken in conjunction with the constancy of boiling point, percentage composition, and molecular weight determination, are conclusive evidence that the substance is nearly pure 2:3-butanediol. The further confirmation of this by actually separating the purified diol from its diphenylurethane is described later (p. 281).

(2) *Oxidation with Semi-normal and Normal Nitric Acid at* 100°.—Among the products of oxidation were found carbon dioxide, acetaldehyde, small quantities of acetylmethylcarbinol and diacetyl, acetic acid, and inactive lactic acid. The last two substances were identified by the composition of their barium and zinc salts respectively:—

Acetic Acid.—

3·535 grm. of barium salt gave 3·1479 grm. of $BaSO_4$.

Equivalent weight of acid is 60·5. Acetic acid, $C_2H_4O_2$ = 60.

Lactic Acid.—

0·4787 grm. crystallised zinc salt gave 0·3908 grm. anhydrous zinc salt and 0·1329 grm. ZnO.

0·1250 grm. crystallised zinc salt gave 0·3908 grm. anhydrous zinc salt and 0·0341 grm. ZnO.

H_2O = 18·36 per cent. ZnO = 34·00 per cent.

$Zn(C_3H_5O_3)_2.3H_2O$ requires H_2O = 18·15 : ZnO = 33·45 per cent.

No oxalate or tartrate was present.

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* v. Pechmann, 'Ber.,' 1888, vol. 21, p. 2755.

† Tsugaeff, 'Ber.,' 1905, vol. 38, pp. 2520—2522.

(3) The crude glycol does not reduce Fehling's solution either in the cold or on heating, though it contains a constituent which reduces ammoniacal silver nitrate* in the cold. This may be removed without any apparent accompanying change to the "crude glycol" by boiling for eight hours with 30-per-cent. caustic soda solution. When the "crude glycol" was boiled at atmospheric pressure for seven hours in contact with air the liquid assumed a brownish tint, which did not increase with time, and $[\alpha]_D$ fell from + 0·723 to + 0·590. On boiling with animal charcoal in aqueous solution and recovering the glycol, the $[\alpha]_D$ was found to be 0·749. No reaction product could be obtained by heating an aqueous solution of "crude glycol" and phenylhydrazine acetate to 100° for one hour.

A mixture of 10 grm. glycol, 32 grm. water, and 8 grm. concentrated sulphuric acid boiled up for 10 hours apparently underwent no change, the material being recovered unaltered.

The above experiments, beyond showing the general chemical stability of the constituents of the "crude glycol," indicated that it was not a pure chemical substance, but did not provide any information as to the other substances accompanying the 2:3-butanediol therein.

Treatment of "Crude Glycol" with Phenylisocyanate, and Fractionation of the Resulting Phenylurethanes.

20·4 grm. of "crude glycol," 64 c.c. phenylisocyanate, 250 c.c. of pure dry ether were boiled on a water-bath for 12 hours till "bumping," due to deposition of crystals commenced. The mixture was then allowed to cool. The supernatant fluid was poured off, and, together with the dry ethereal washings of the crystals, boiled up again for an additional seven hours. This liquid was concentrated, and, on cooling, the mother liquors containing the excess of phenylisocyanate were removed, and the isocyanate decomposed by alcohol. In some later preparations the excess of phenylisocyanate, together with the most soluble phenylurethanes, was carried on to the next batch. Precautions were taken to prevent access of water during the performance of the above operations. The fractionation of the reaction-products was commenced by recrystallisation from alcohol. Four substances were obtained pure in this way. The mother liquors containing substances more soluble in alcohol were dissolved in benzene, and precipitated fractionally by adding light petroleum.

Altogether, about 105 grm. of "crude glycol" were treated with phenylisocyanate, corresponding to about 383 grm. of mixed phenylurethanes.

From alcohol there were obtained:—

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* Tollens, 'Ber.,' vol. 15, p. 1635.

(1) 2 : 3-*Butyleneglycoldiphenylurethane.*—It is sparingly soluble in cold alcohol, ether and benzene, crystallises in rosettes of colourless needles and melts at 199°·5 (uncorr.). One hundred cubic centimetres of a solution in absolute alcohol saturated at 13° contain 1·02 grm. No evidence of optical activity was found in the pure material. Its hydrolysis will be described later (p. 280). It comprises about 90 per cent. of the whole amount of the reaction-product.

0·2783 grm. gave 0·6716 grm. CO_2 and 0·1557 grm. H_2O.
0·2725 grm. gave 20·2 c.c. N at 23°·5 and 766 mm.

	Found.	Calculated for $C_4H_{10}O_2(C_6H_5CNO)_2$
C	65·79	65·85
H	6·21	6·09
N	8·57	8·53

(2) 2 : 3-*Butyleneglycoldiphenylurethane and Diphenylurea in the Proportion* $C_{13}H_{12}ON_2.2C_{18}H_{20}O_4N_2$.—When the mother liquors from (1) were concentrated, further crops of crystals were obtained, which, recrystallised from hot alcohol, gave colourless prisms very similar in physical properties to (1), and melting at 187°·5 C. Repeated recrystallisation from alcohol did not alter this melting-point. By the use of benzene as a solvent, however, careful fractionation of this material yielded crops melting at 235° (diphenylurea) and 196° (diphenylurethane of 2 : 3-butyleneglycol) respectively. On taking pure specimens of these two substances and dissolving them in hot alcohol there was deposited again this complex, melting at 187°·5.

On analysis the following figures were obtained:—

I. 0·1993 grm. gave 0·4923 grm. CO_2 and 0·1110 grm. H_2O,
II. 0·1745 grm. „ 0·4320 grm. CO_2 and 0·0994 grm. H_2O.
I. 0·2809 grm. „ 23·6 c.c. N (moist) at 24°·5 and 765 mm.
II. 0·2096 grm. „ 16·8 c.c. N (moist) at 23° and 764 mm.

	Found.		Calculated for $C_{49}H_{52}N_6O_9$.
	I.	II.	
C	67·37	67·52	67·7
H	6·20	6·33	6·00
N	9·48	9·25	9·67

These facts, taken together, indicate that these crystals had the composition

$C_{13}H_{12}ON_2$	+	$2(C_{18}H_{20}O_4N_2)$	=	$C_{49}H_{52}N_6O_9$
Diphenylurea (m. pt., 235°).		Diphenylurethane of 2 : 3-butyleneglycol (m. pt., 199°·5).		Complex (m. pt., 187°·5).

The formation of the diphenylurea is doubtless partly due to moisture gaining access to the reaction mixture and present in the "crude glycol."

(3) 2 : 3-*Butyleneglycolmonophenylurethane.*—In later preparations, the formation of the substance was avoided by using excess of phenylisocyanate and continuing the heating for many hours. It resembles the diurethane very closely in its solubility relationships, but it is, of course, more soluble than this. The melting point of the pure substance recrystallised from alcohol was found to be 100° C., and a sample of the material treated with phenylisocyanate in benzene solution at its boiling point for eight hours gave an almost theoretical yield of the diphenylurethane (melting point, 199°).

0·0808 grm. gave 0·1876 grm. CO_2 and 0·0572 grm. H_2O.
0·2558 grm. „ 15·00 c.c. N (moist) at 16° and 766 mm.

	Found.	Calculated $C_{11}H_{15}NO_3$.
C	63·32	63·16
H	7·86	17·7
N	6·89	6·65

1·2 grm. of material in 57 c.c. of benzene gave in a 200-mm. tube a rotation of +0°·004. If it is assumed that a rotation of 0°·01 is detectable with certainty, the specific rotation for this substance in benzene solution cannot be greater than +0°·25.

Fractionation by means of Benzene and Petroleum.—The alcoholic mother liquors from the three substances described were now concentrated and taken up in benzene. A collection of a quantity of this material was made, and then fractionation effected by precipitation with low boiling-point petroleum ether. After a while the mother liquors became thick, and then the phenylurethane formed by the reaction of phenylisocyanate and the alcohol added was removed by distillation *in vacuo*. The distillate, boiling at about 120° under reduced pressure, consisted of nearly pure phenylurethane, melting after recrystallisation at 49°·5. The value given by Paal and Otten* is 52°.

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* Paal and Otten, 'Ber.,' vol. 23, p. 2590.

The residue from the distillation was dissolved in benzene, and fractionation by petroleum ether was continued. It became necessary at this stage to take the $[\alpha]_D$, as well as the melting point and weight of each crop, and these crops were then sorted out with respect to their optical activities. It frequently happened that when material that was considered inactive was further fractionated it yielded strongly dextro- and strongly lævo-rotatory substances. The separation at each step was very slight, and a very large number of precipitations was required before any fractions which could be regarded as pure substances were obtained.

The final products of this fractionation of the substances contained in the alcoholic mother liquors mentioned above were:—

(1) A substance of $[\alpha]_D$ about -50 in alcoholic solution, melting at 145° C. The glycol obtained by hydrolysis was strongly dextro-rotatory.

(2) A substance of $[\alpha]_D$ greater than $+34$ and melting higher than 200° C.

These substances were not further examined, owing to the small amount obtainable in the pure condition, as judged by the definiteness of the melting point. One of them is possibly the diphenylurethane of an active 2 : 3-butanediol.

(3) A substance melting at 157°, and optically inactive in alcoholic solution. Analysis:—

0·1056 grm. gave 0·2530 grm. CO_2 and 0·0578 grm. H_2O.
0·1120 grm. „ 8·6 c.c. N. at 12° and 723 mm.

	Found.	Calculated for $C_4H_{10}O_2(C_6H_5CNO)_2$.
C	65·3	65·82
H	6·08	6·09
N	8·62	8·53

0·3 grm. was taken and hydrolysed in the manner described below and the aqueous solution of the resulting glycol oxidised by bromine in the light. On distillation under a long column the characteristic yellowish-green ring of diacetyl was seen to come over and the material was found to possess the peculiar smell of diacetyl. The distillate reduced Fehling's solution at once in the cold and yielded a crystalline osazone. The quantity was so minute that a melting-point determination could not be performed satisfactorily.

The evidence obtained, however, was considered to prove that the inactive diphenylurethane melting at 157° gave on hydrolysis a butanediol which yielded diacetyl by oxidation with bromine in the light and was therefore the diphenylurethane of a 2 : 3-butanediol.

Recovery of 2 : 3 *Butanediol from Purified Diphenylurethane, melting at* 197° *to* 199°.

(1) A quantity of the inactive purified diphenylurethane of 2 : 3 butanediol of melting point 197° to 198°·5 (p. 278) was boiled up with alcoholic caustic potash until a small quantity gave no precipitate with excess of water. Carbon dioxide was then passed in to saturation, the solution evaporated to dryness *in vacuo*, and the glycol dissolved out in alcohol. The alcoholic solution on distillation gave a yellow oily liquid. This remained yellow on redistilling several times, ultimately, however, by repeated boiling with animal charcoal it was obtained colourless. Two combustions of this substance were made—

I. 0·2449 grm. gave 0·4746 grm. CO_2 and 0·2396 grm. H_2O.
II. 0·3592 grm. „ 0·6945 grm. CO_2 and 0·3550 grm. H_2O.

	Found.		Calculated for $C_4H_{10}O_2$.
	I.	II.	
C	52·85	52·75	53·33
H	10·87	10·99	11·11

The boiling point (uncorr.) at atmospheric pressure was 180° to 181°.

(2) In a second preparation 2·35 grms. were recovered from 14 grms. of diurethane.

		Found.
0·2322 grm. gave 0·4497 grm. CO_2 and 0·2298 grm. H_2O	C	52·82
	H	10·99

This glycol showed a very faint but just recognisable positive rotation.

(3) A sample of diphenylurethane (melting point 199°) had been many times recrystallised with great care until in the mother liquors, when brought to maximum concentration, no optical activity could be observed. Of this 29·1 grm. were hydrolysed as before. 4·1 grm. of 2 : 3-butanediol were obtained. The value of $[\alpha]_D$ in 10 per cent. dry ether solution was demonstrated to be less than +0·08 and it was considered that this material was the inactive substance corresponding to the formula—

$$CH_3.CH(OH).CH(OH).CH_3.$$

At ordinary temperatures it exists as a hard white mass.

0·2432 grm. gave 0·4691 grm. CO_2 and 0·2424 grm. H_2O.

	Found.	Calculated $C_4H_{10}O_2$.
C …………	52·6	53·33
H …………	11·07	11·11

It will be seen that the combustions of the "glycol" prepared from the diphenylurethane consistently give figures for the carbon content which are less than the theoretical. This cannot be put down to errors in the combustion process. It is suggested that a small percentage (about 1 per cent.) of water persistently accompanies the glycol even in its distillation. A sample treated in aqueous solution with bromine in the light gave diacetyl from which the osazone of melting point 241° to 243° was prepared. The identity of this osazone with the corresponding one from the "crude glycol" was established. With iodine and potash an aqueous solution of the glycol gives an immediate precipitate of iodoform.

It is seen that the "crude glycol" has given large quantities of a pure diphenylurethane from which a pure butanediol has been prepared by alkaline hydrolysis. This recovered diol yields diacetyl on oxidation, and this shows conclusively that the butanediol has a straight chain with the two hydroxyl groups in the 2 : 3-position. On this, and the fact of the large yield of diphenylurethane melting at 199°·5 when phenylisocyanate acts on the "crude glycol" is based the conclusion that well over 90 per cent. of the "crude glycol" consists of an inactive 2 : 3-butanediol. From the remaining 10 per cent. only one substance has been definitely isolated and this appears also to be an inactive 2 : 3-butanediol, but yields a phenylurethane melting at 157°. It is probable that one of these inactive diols is the *r.* or *d.l.* isomer and the other the internally compensated isomer, but no attempt has been made to decide this question.

II. THE EFFECT OF FREE OXYGEN ON THE ACTION OF *B. lactis aërogenes* ON GLUCOSE AND ON 2 : 3—BUTANEDIOL.

In the experiments described the amount of acetylmethylcarbinol obtained from 50 grm. of glucose was very small and it was desired to increase the yield of this substance. An endeavour to do this by passing oxygen into the inoculated flask during incubation proved successful. It was also found that acetylmethylcarbinol is abundantly formed when the bacillus is cultivated in a solution of the butanediol in 1 per cent. peptone water in a current of oxygen. The oxidation of an alcohol group to a ketone group by an organism in an abundant supply of oxygen is interesting, and is quite analogous to the bacterial oxidation of mannitol to fructose (Vincent and Delachanal),* sorbitol to sorbose, and glycerol to dihydroxyacetone (Bertrand).†

The Arrangement of the Experiments.

Two similar flasks were filled with the same medium.

$$\text{In Experiment I} \ldots\ldots \left\{\begin{array}{l}\text{Glucose, 50 grm.}\\ \text{Witte peptone, 10 grm.}\\ \text{Chalk, 15 grm.}\end{array}\right\} \text{Water to 1 litre.}$$

$$\text{In Experiment II} \ \ldots \left\{\begin{array}{l}\text{Butyleneglycol, 8 grm.}\\ \text{Witte peptone, 10 grm.}\end{array}\right\} \text{Water to 1 litre.}$$

One, after sterilisation, was freed completely from oxygen in the manner described,‡ inoculated, fitted with a mercury trap, and incubated at 37° for 21 days.

The other, also after sterilisation, was inoculated and incubated at 37° for 22 days, but a stream of oxygen from a cylinder fitted with a reducing valve, after passing through two wash bottles containing water and kept at 37°, was bubbled through it the whole time at the rate of about 50 c.c. per minute. The volume of the contents of the flask scarcely changed during the passage of the gas. The oxygen after leaving the flask passed again through wash bottles kept cold by circulation of the main supply (12° C.) and up an inverted condenser. Any acetylmethylcarbinol carried over by the oxygen was thus trapped. In Experiment I the proportion of carbon dioxide in the gas leaving the apparatus never exceeded 2 per cent.

The Examination of the Media after Fermentation.

Experiment I.—The glucose in the residues after distillation was determined by Pavy's method after removal of protein by mercuric nitrate and excess of mercury by caustic soda. The residual sugar in the anaërobic culture was less than 3 grm.; that in the aërobic culture was less than 0·6 grm.

The comparison of the amounts of acetylmethylcarbinol produced in the two cases was conducted as follows. The contents of the two flasks were filtered from unattacked $CaCO_3$ and insoluble calcium salts and the filtrates distilled until in each case 800 c.c. of distillate were collected. Very small traces of acetylmethylcarbinol can be detected by Fehling's solution, and previous experience with this substance demonstrated that such a distillate would contain nearly all the acetylmethylcarbinol of the medium. A number of experiments were then made to determine the volume of distillate in each case which, when made up to 10 c.c. with distilled water and 1 c.c. Fehling's solution, *just* brought about complete reduction in 10 minutes. This was found to be :—

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* 'Compt. Rend.,' 1897, vol. 125, pp. 716—717.

† 'Ann. Chim. Phys.,' 1904, 18, vol. 3, pp. 181—288.

‡ 'Jenner Inst. Trans.,' 1899, vol. 2, p. 129.

Aërobic flask	0·7 c.c.
Anaërobic flask	3·9 „

There was no other volatile reducing substance discovered in these fermentation products, nor was there any indication of a foreign substance which would vitiate, by its presence, the accuracy of these results. The amount of acetylmethylcarbinol produced in the aërobic fermentation was therefore $\frac{3·9}{0·7} = 5·6$ times that produced in the similar fermentation under anaërobic conditions.

The acetylmethylcarbinol was found to be lævo-rotatory, but was not obtained pure enough for a determination of the $[\alpha]_D$.

Experiments with synthetic acetylmethylcarbinol (kindly made by Mrs. D. Norris) show that under the above conditions 1 c.c. of Fehling's solution is reduced by 2·48 mgrm. of the carbinol. The absolute quantities found were therefore approximately,

Aërobic flask	$\frac{800 \times 2·48}{0·7} = 2·83$ grm.
Anaërobic flask	$\frac{800 \times 2·48}{3·9} = 0·51$ „

The extraction of crude 2 : 3-butanediol (boiling point, 181 to 183°) from the two residues of the two cultures was performed in precisely the same manner in each case. In the anaërobic experiment 7·9 grm., and in the aërobic experiment 6·85 grm. were obtained. The values of $[\alpha]_D$ in 10-per-cent. solution in dry ether for these two samples were found to be +1·71 and +1·52 respectively.

Experiment II.—The culture made under anaërobic conditions did not grow. The stopper was therefore removed, the flask reinoculated and the stopper replaced, and the flask was then incubated. Growth then took place.

After the expiration of three weeks both flasks were found to contain pure cultures of *B. lactis aërogenes.* The liquids were distilled exactly as described for Experiment I, and the reducing power to Fehling estimated. This was found to be, for 1 c.c. Fehling solution—

Anaërobic flask	22·4 c.c. approx.
Aërobic flask	3·6 „

The acetylmethylcarbinol was identified by conversion into the osazone of diacetyl.

The relative amounts produced anaërobically and aërobically from 2 : 3-butanediol were therefore as $1 : \frac{22 \cdot 4}{3 \cdot 6} = 1 : 6 \cdot 2$, which is, curiously enough, nearly the same ratio as that found to exist between the corresponding amounts from glucose, 1 : 5·6. The absolute amounts were :—

Aërobic flask............	0·55 grm.
Anaërobic flask.........	0·09 „

III. The Action of *B. lactis aërogenes* on Fructose.

One litre of medium was made up to contain 1 per cent. of peptone, 1·5 per cent. calcium carbonate, and 5·0 per cent. of fructose. The period of incubation at 37° was 37 days. After filtration the volume was 963 c.c., and from this 750 c.c. were distilled. The distillates readily reduced Fehling's solution in the cold, and when extracted with ether after saturation with potassium carbonate a liquid was obtained which gave with phenylhydrazine acetate an osazone melting at 240° C. It was therefore concluded that acetylmethylcarbinol was present. The residue was treated in the manner described for glucose, and the weight of crude glycol obtained was 3·2 grms. The value of $[\alpha]_D$ was 1·32 in 10-per-cent. solution in dry ether.

During the course of this investigation the following substances, in addition to those described, were prepared for purposes of comparison :—

1. *Diphenylurethane of* 1 : 3-*butanediol* was obtained from the diol prepared by the reduction of aldol. It crystallises readily from alcohol in colourless prisms, melting at 122° to 123° C.

0·2763 grm. gave 20·2 c.c. N at 14°·8 and 768 mm.

	Found.	Calculated for $C_{18}H_{20}N_2O_4$.
N	8·77	8·53

2. *Diphenylurethane of* 1 : 2 *Propanediol.*—The glycol from which it was prepared boiled at 185° to 195° at ordinary pressure. The diphenylurethane is readily soluble in hot alcohol, from which it is for the most part precipitated in colourless silky needles on cooling. These melt at 152°·5 to 153°·5.

0·2571 grm. gave 0·6138 grm. CO_2 and 0·1306 grm. H_2O.

0·2581 grm. „ 19·7 c.c. N (moist) at 770 mm. and 20° C.

	Found.	Calculated for $C_{17}H_{18}N_2O_4$.
C	65·11	64·96
H	5·64	5·73
N	9·01	8·91

My thanks are due to Dr. Harden for advice throughout the investigation.

Summary.

1. The "crude glycol" obtained by the action of *B. lactis aërogenes* on glucose contains two optically inactive 2:3-butanediols, whose diphenylurethanes melt at 199°·5 and 157° respectively. The former constitutes well over 90 per cent. of the material. As no attempt was made to resolve either, it is impossible to say which is the racemic and which the meso- form.

2. Small quantities of the phenylurethanes of other glycols of unknown constitution were obtained from the crude glycol.

3. Acetylmethylcarbinol is formed from 2:3 butanediol by *B. lactis aërogenes* in presence of oxygen, and the yield from glucose is increased by the presence of oxygen.

4. Fructose is decomposed by the bacillus in a similar manner to glucose.

The Pharmacological Action of South African Boxwood (Gonioma kamassi).

By W. E. Dixon.

(Communicated by Prof. J. N. Langley, F.R.S. Received November 1, 1910,—
Read January 19, 1911.)

(From the Pharmacological Laboratory, Cambridge.)

The boxwood products with which the following experiments were performed were prepared from the wood of *Gonioma kamassi* exported from Knysna and belonging to the N.O. Apocynaceæ. The wood was carefully identified and sent in sealed parcel to Mr. E. F. Harrison, who extracted an alkaloid. The certain identity of the wood is of some importance, as the preparations which were used in my experiments cannot be called into question and as there has been some confusion in the past between *Gonioma kamassi* (South African box), *Buxus macowani* (East London boxwood) and *Sarcocephalus diderrichii* (West African boxwood).

The action of South African boxwood has assumed an economic importance in recent years, since it has on occasion and in limited amount been substituted for Persian and other boxwoods in the manufacture of shuttles in Lancashire, and it is alleged that symptoms of poisoning have arisen in a percentage of the workmen employed in sawing this wood or in finishing the chiselled shuttles. The symptoms complained of seem to vary a good deal in different individuals, but respiratory trouble, sometimes of a nature simulating spasmodic asthma, headaches, cerebral depression, and nasal catarrh appear to be the most frequent.

Harvey Gibson* extracted from an African boxwood—probably *G. kamassi* though the wood was not definitely identified—0·07 per cent. of an impure alkaloid; with this he performed some perfusion experiments on the isolated mammalian heart; from these he concludes that the alkaloid is a cardiac poison.

However valuable experiments on isolated organs may be in conjunction with other experiments, taken alone they teach little. The known action of a number of substances on isolated muscle or heart gives no indication of their relative toxicity to the normal intact animal. And although it is easy enough to poison an isolated perfused heart with boxwood, as it is with almost any chemical substance, yet it is not possible to depress seriously the heart in an intact animal, since before the heart is even appreciably affected respiration ceases and death occurs from asphyxia. Boxwood has no specific affinity for cardiac or other muscle.

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* 'Biochemical Journ.,' 1906, vol. 1, p. 39.

The two samples of alkaloid I used, Mr. Harrison informs me, were prepared as follows:—

"A" was prepared by heating the sawdust with dilute hydrochloric acid, straining and pressing; the acid liquid was then concentrated, filtered from the resinous matter which separated, and precipitated by potassium bismuth iodide solution; the precipitate was decomposed by sodium hydroxide and the alkaloid extracted by alcohol; the alcoholic solution was evaporated, the residue extracted with dilute hydrochloric acid, leaving resin undissolved, and the alkaloid liberated from the solution by ammonia and shaken out with chloroform; the alkaloid was further freed from resin by repeating the treatment with acid, ammonia, and chloroform. When finally converted into hydrochloride the solution deposited a little more resin on standing, which was filtered off.

"B" was prepared by percolating the sawdust with alcohol, evaporating the alcoholic liquid, and taking up the residue with dilute hydrochloric acid, leaving resin; the acid solution was then precipitated with phospho-tungstic acid, the precipitate decomposed with sodium hydroxide and the alkaloid extracted by alcohol; the subsequent treatment was the same as for "A." The experiments were performed on cats, dogs, frogs, and rabbits, which, unless otherwise stated, were anæsthetised with A.C.E. mixture and later with urethane.

Effect on the Circulation.—The injection of small doses of the alkaloid, from 10 to 20 milligrammes, into the circulation of the dog or cat causes a rapid and considerable fall of blood-pressure; the pressure rises again slowly, but never to its former height. Further injections produce less and less effect on blood-pressure until, after 40 or 50 milligrammes in the case of the cat, a point is reached when further injections are without decided influence (fig. 1).

A fall of blood-pressure of this nature is characteristic of the group of drugs which paralyse autonomic nerve cells, such as curare, apocodeine, coniine and the like, and should be entirely vaso-motor in origin. That there is considerable vaso-dilatation of the splanchnic area can be readily shown either by the oncometer or the method of perfusion. This may be inferred from the increased volume pulse of the intestinal vessels shown in fig. 4B. In plethysmographic tracings it is clear that the fall of blood-pressure corresponds fairly accurately with the degree of dilatation.

Perfusion experiments in which oxygenated Ringer's solution is forced at constant pressure through the intestinal or limb vessels of a rabbit also show

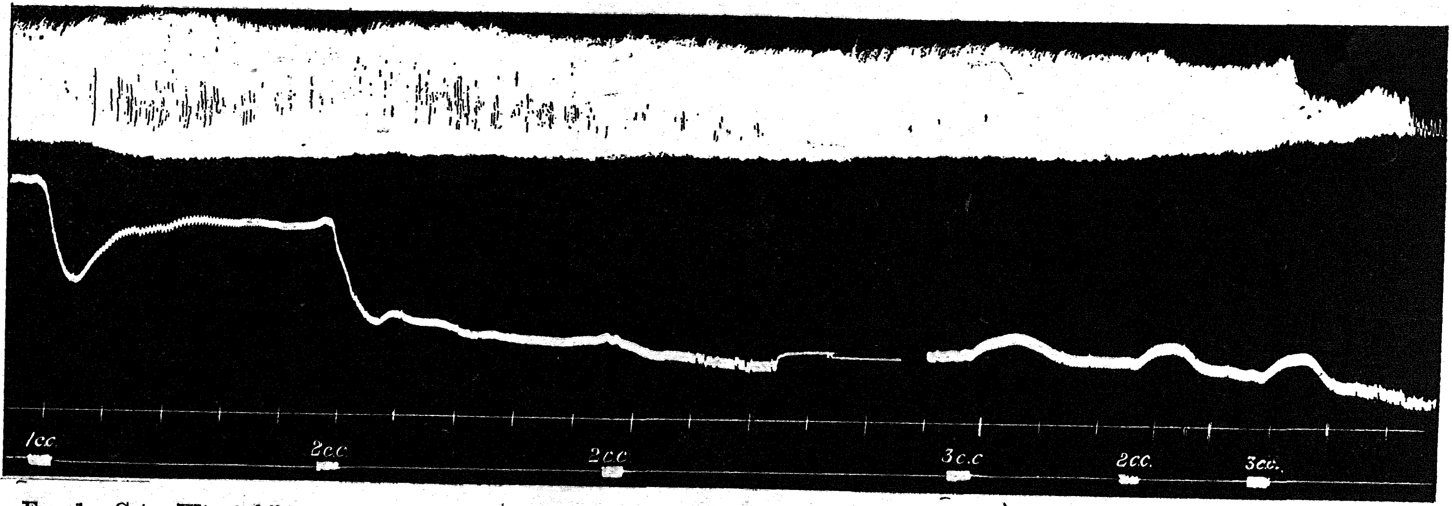


FIG. 1.—Cat. Wt., 2 kilogrammes. A.C.E., urethane. Upper curve is respiration, upstroke representing inspiration. Lower curve is blood-pressure, taken with a mercurial manometer. Time, half minutes. Total injection of alkaloids, 65 milligrammes.

the dilatation of vessels. In these experiments the Ringer's solution at body temperature was suddenly changed to one containing 0·01 per cent. of the alkaloid, when the outflow from the vein showed a decided increase. It is clear, therefore, that one cause of the fall in blood-pressure is vaso-motor. It has been suggested, however, by Harvey Gibson from observations on the isolated heart of the rabbit that boxwood is a profound cardiac poison. His experiments on the isolated heart are easily verified (fig. 2), but of course such experiments do not show that, in any possible dosage to a normal animal, the amount present in the blood would be sufficient to exert this influence on the action of the heart. Indeed, the amount of boxwood which must be administered to an isolated heart in order to produce depression also very decidedly influences its nerve supply, to which later reference will be made; hearts so treated will be found to have lost their reaction towards muscarine and pilocarpine, whilst towards adrenalin their reaction is much diminished. In order to decide whether boxwood has any cardiac action of importance, several experiments were made on the intact heart of anæsthetised animals. The results of these experiments showed in each

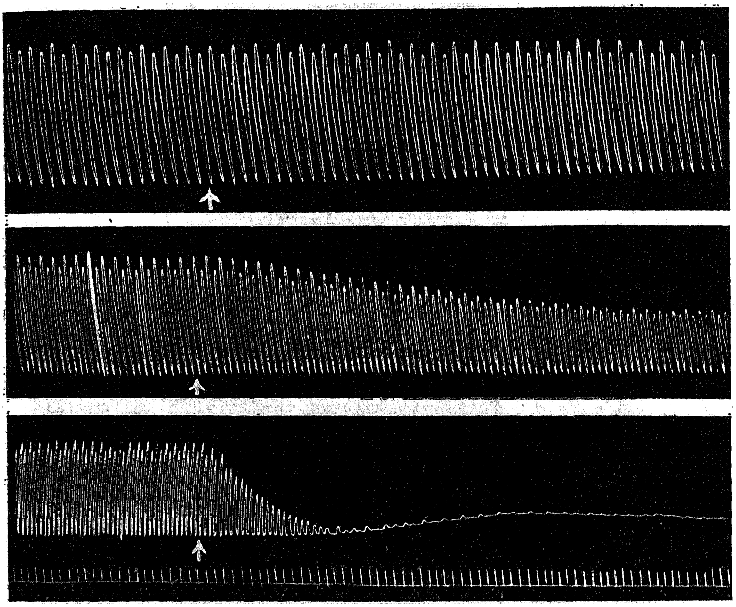


Fig. 2.—Record of Isolated Rabbit's Heart perfused with Oxygenated Ringer's Solution. The top curve shows the effect of injecting 1 c.c. of a 0·04-per-cent. solution of the alkaloid; the middle tracing shows the effect of injecting 5 c.c. of the same strength, and the bottom curve 10 c.c. of a 0·08-per-cent. solution. Time, seconds. Each injection was made in 5 seconds.

case that the heart was not appreciably influenced, even when doses of boxwood were given sufficient in amount to paralyse completely the brain and medulla. From experiments with the cardiometer (fig. 3) it is obvious that the administration of the drug is followed by a period in which the output of blood from the heart per minute is actually greater than the normal, and in no case has a diminution in the output been observed. It will be seen from this figure, which is quite typical, that it is the strength of the systole which is especially increased, and that the effect is prolonged.

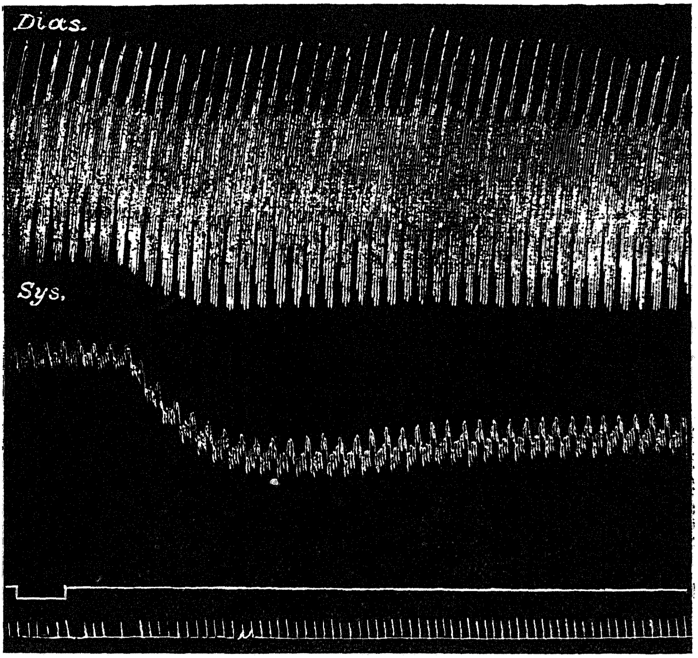


Fig. 3.—Cat. Urethane. Cardiometer and blood-pressure. Shows the effect of injecting 10 milligrammes of alkaloid. Note increased cardiac output and augmentation of systole. Time, seconds.

At first sight it might seem that these experiments with the isolated heart and with the cardiometer are contradictory, but such is not the case, since any drug which produces a pure vaso-dilatation causes secondary effects on the heart, which result in an augmented output of blood per minute; thus nitrites depress the isolated heart, as indeed they do all muscle tissue, yet when taken by an intact animal the cardiac output is increased. All the isolated heart experiments teach is that boxwood exerts no direct stimulant action on cardiac muscle. Even when natural respiration has ceased in an animal as a direct result of boxwood poisoning, additional doses of the alkaloid may still be given without causing any appreciable depression of the heart. The effects described are the same in the frog, rabbit, cat, and dog, so that it can hardly be argued that direct cardiac failure is a serious feature in poisoning by boxwood.

Respiration.—If records be taken of the respiratory movements, it will be found that the effect of boxwood is to cause at first a gradual increase in the depth of respiration without much alteration in the rate. This effect can be seen quite well by giving a small injection, 8 or 10 milligrammes, to a normal cat and observing without the use of any recording apparatus. Figs. 1 and 6 show the effect of the poison recorded graphically; in each case the initial action of boxwood on respiration is some increase in depth, the volume of air inhaled at each breath is greater, whilst the rate remains about the same. As more drug is administered this primary stimulant action is quickly followed by depression, as shown by the diminution in respiratory movements, and if the dose be sufficiently large, respiration is completely paralysed. An effect of this nature is seen in fig. 1, after the administration of 65 milligrammes of boxwood alkaloid into the circulation of a cat, and in fig. 6 after an injection of 30 milligrammes. In all cases the respiration having once ceased never returned, so far as the length of the experiment, sometimes extending for three hours, allowed of observations being taken.

With an active circulation such a failure of respiration may be due either to central paralysis of the medulla or to peripheral motor paralysis. If the motor nerves of respiration, the phrenics and intercostals, be excited with a tetanising current immediately natural respiration has ceased, they are found to cause contraction of their respective muscles, though the strength of contraction is considerably diminished from the normal; still, no doubt, ample activity remains for some kind of respiratory movements to occur were a powerful stimulus to proceed from the centre. At this stage, for example, strychnine still induces violent muscular twitchings. Electrical stimulation of the medulla, however, causes no respiratory movement, and the administration of hydrocyanic acid, a drug which powerfully excites the respiratory centre, is without effect. At this stage it is also interesting to note that in the rabbit the cardiac vagus is still active, whilst in the cat it is generally completely paralysed.

Action on Nerve-cells—Central Nervous System.—The administration of moderate non-poisonous doses of the boxwood alkaloid to all normal animals causes increased activity of the reflexes. The effect is one apparently upon the spinal cord, since in the frog it is still persistent after complete destruction of the brain. In anæsthetised animals, the effect may also frequently be seen for a short period before the depression of the motor nerves is pronounced, thus it may be observed after the injection of 30 or 40 milligrammes of the alkaloid into cats; sometimes, indeed, the reflexes may be so increased as to induce a series of convulsive jerks. The tendency to such movements is naturally more pronounced after the administration of the drug to decerebrate rather than to anæsthetised animals. True tonic and clonic convulsions are never observed. This action on the spinal cord is no doubt partly masked by the depressant action of the drug on the cerebrum and the motor nerve endings; but these effects can be to some extent eliminated, at least for a time, by injecting the drug directly into one of the veins of the spinal cord, and in such cases typical strychnine-like convulsions occur. Depression of the higher cerebral centres appears to occur simultaneously with the stage of exaggerated spinal reflexes. Normal animals injected with this alkaloid may show all the effects of cerebral depression at a time when the spinal reflexes are much increased.

As in the case of curare, apocodeine and coniine, therefore, three effects can be analysed which to some extent mask one another: (1) depression of the cerebrum, (2) "stimulation" of the spinal cord, (3) paralysis of the motor nerve-endings. By appropriate means each of these effects can be observed with all three drugs, but whereas with boxwood all three are produced nearly simultaneously with a certain dose of drug, with curare the motor nerve-endings are paralysed first, or about the same time as the central nervous system, and completely mask the convulsions, whilst with coniine and apocodeine the paralysis of the central nervous system is the marked feature, and the motor nerve-endings are only paralysed after the administration of very much larger doses of alkaloid.

Vagus.—The injection of boxwood causes paralysis of the vagus in all mammals. In the rabbit, complete paralysis is not attained until the respiration has ceased; but in the cat, vagal paralysis precedes respiratory. Thus in a cat of 2 kilos., electrical stimulation of the vagus nerve ceased to inhibit the heart after the administration of 30 milligrammes of the alkaloid. Immediately paralysis is complete, muscarine, and in a less marked degree pilocarpine, still cause inhibition, although if a somewhat larger amount of the boxwood is given the paralysis is made as complete as by atropine, and muscarine is now quite without action on the cardiac rhythm. The usually accepted explanation of phenomena of this description is that the nerve-cells are paralysed a little before the nerve-endings, and the boxwood paralyses the nerve-cells whilst the post-ganglionic fibres are for a time still intact, as shown by their response to muscarine.

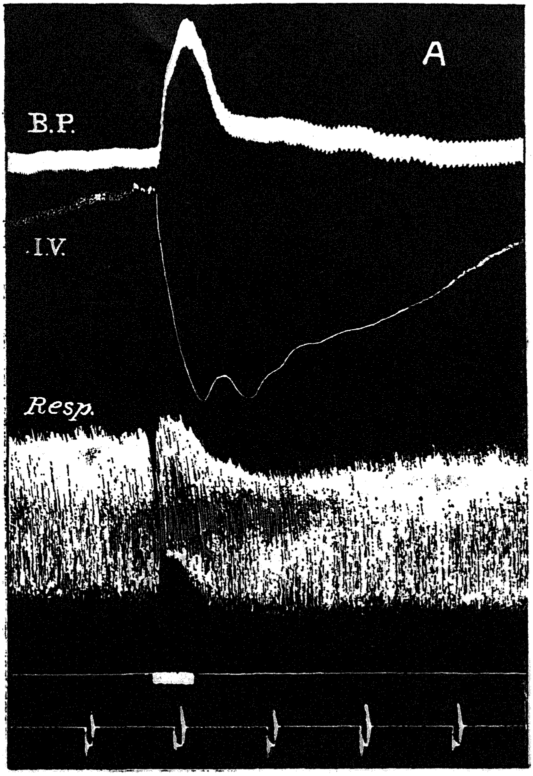
Sympathetic Nerves—(a) *Eye.*—Quite small doses of the boxwood alkaloid, such as 20 milligrammes, injected into the circulation of the rabbit or cat, paralyse the sympathetic ganglion cells of the eye before the respiration has been appreciably affected. Thus, in one experiment on a small cat, it was found that well-marked dilatation of the pupil and opening of the eyelid resulted from stimulation of the sympathetic nerve with the secondary coil at 12 cm. A slow injection of 20 milligrammes of the alkaloid as a 0·5-per-cent. solution was made into the jugular vein and this caused paralysis, so that, a few minutes later, stimulation of the sympathetic, with the secondary coil at 5 cm., failed to elicit a response in the eye. Post-ganglionic stimulation, however, produced a typical effect with the coil at 12 cm.

(b) *Splanchnic.*—Boxwood paralyses the ganglion cells in the course of the splanchnic nerve, in the same way as it paralyses the cells in the course of the sympathetic nerve to the head. The injection of from 30 to 50 milligrammes of boxwood alkaloid into the circulation of a cat blocks the conduction of impulses through the splanchnic nerve, so that its electrical excitation is without influence on the blood vessels. Stimulation of the post-ganglionic fibres still causes typical constriction of the splanchnic vessels, with a resulting rise in systemic blood-pressure (fig. 4). When still larger doses of the alkaloid are injected, a further stage is reached in which even excitation of post-ganglionic fibres is without effect. These facts may be interpreted as meaning that the ganglion cells are first poisoned, and later some portion of the peripheral nervous mechanism; for it is certain that, even after the most complete paralysis, the muscle fibres of the vessels still respond to muscle poisons, such as lead, barium, veratrine, squill, and the like.

Effect on Motor Nerve-endings.—Large doses of boxwood paralyse the motor nerves in a manner similar to curare; in the case of the cat 80 milligrammes injected intravenously are generally sufficient to cause complete paralysis to electrical stimulation, and smaller amounts effect the same result in the rabbit. The frog is peculiarly resistant and relatively large doses are required to induce this paralysis; in this animal the central nervous system is paralysed easily enough, but the effect on the motor nerves is delayed. Strychnine in any dosage administered to animals in which the motor nerves have been paralysed has, of course, no effect on the muscles, nor can the spasmodic twitchings characteristic of physostigmine poisoning be obtained in such animals. The muscles, however, are intact and respond readily to all ordinary stimuli.

Effect of Certain Drugs. Nicotine.—Evidence has been given already to show that boxwood acts upon the same structures as nicotine, so that were nicotine injected into an animal already under the influence of boxwood it should produce none of its characteristic stimulant effects. Injections of

4A.



4B.

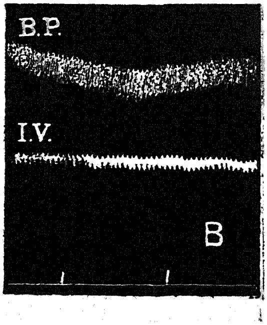


FIG. 4.—Cat. Weight 1400 grammes. B.P., Intestinal Volume and Respiration. A shows the effect of splanchnic excitation with the secondary coil at 15 cm. Tracing B shows the B.P. and intestinal volume 15 minutes later, after the injection of 45 milligrammes of alkaloid. Splanchnic excitation with the coil pushed home is now without effect. Note the increased volume pulse in B as evidence of vaso-dilatation of splanchnic vessels. Time, half-minutes.

nicotine are without appreciable effect when injected under these conditions (fig. 5). Adrenalin, on the contrary, retains its action, feebler than usual it is true, but still characteristic. But very large doses of boxwood can eliminate even the adrenalin effect on the vessels and heart. Pituitary extract also retains its pressor action, as well as the true muscle poisons, lead, barium, and digitalis.

Some Apparent Stimulation Effects.—Boxwood has now been shown to have a depressant effect on most nerve tissues, but one of the effects of small doses is to increase the movements of plain muscle in the body. This is easily shown in the case of the alimentary canal or uterus by the intravenous injection of small doses. Thus fig. 6 is the record of an experiment

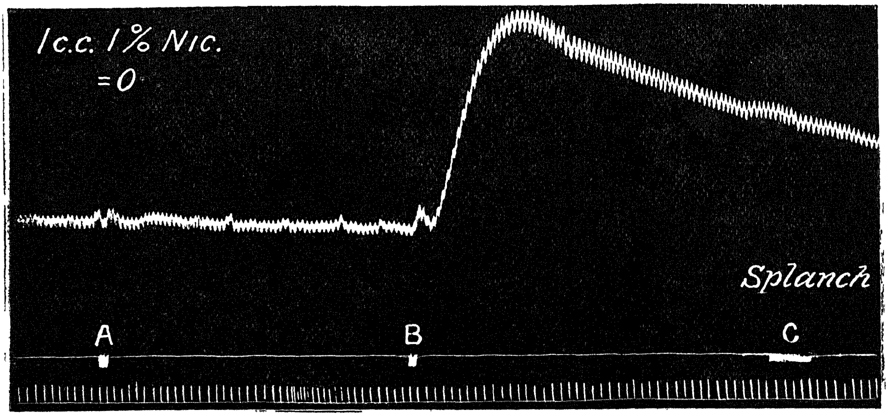


Fig. 5.—Cat. A.C.E. Blood-pressure Curve after the Administration of 55 milligrammes of Alkaloid. At A 1 c.c. of a 1-per-cent. solution of nicotine was injected, and at B 1 c.c. of a 1-in-4000 solution of natural adrenaline. At C the splanchnic nerve was excited by a strong tetanising current. Time, seconds.

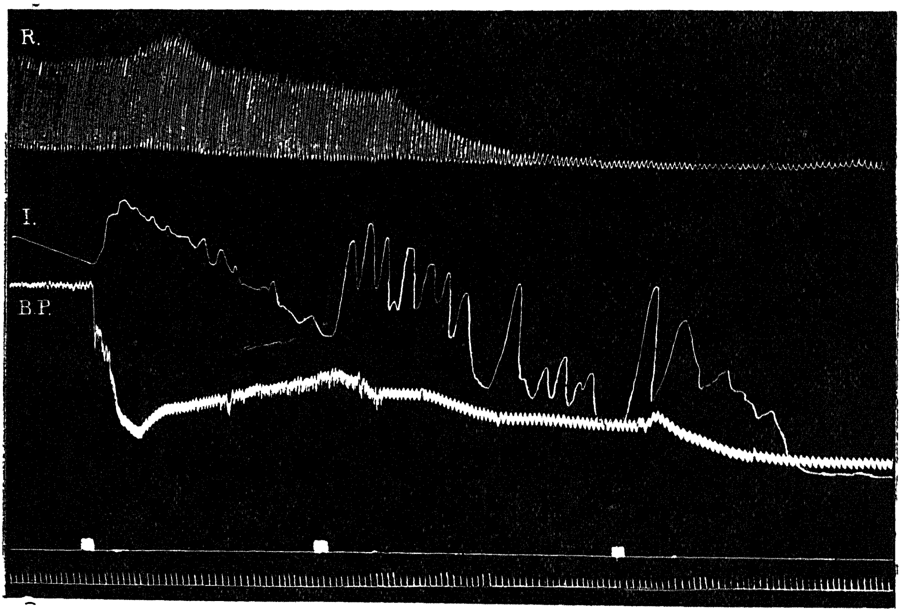


Fig. 6.—Cat. A.C.E. Respiration. Intestinal Movements recorded by a Balloon. B.P. 10 milligrammes of alkaloid injected at each of the indicated marks. The bowel was paralysed after the third injection, though the muscle still responded to mechanical stimulation and muscle poisons. Time, seconds.

in which the pendulum movements of the intestine are recorded; each injection of boxwood is followed by active peristaltic movements until after 40 milligrammes have been given, when the intestine is paralysed and further injections are without effect; at this stage muscarine and pilocarpine, which normally evoke violent peristaltic movements usually regarded as vagal stimulation, fail to have an action, though the muscle still contracts to mechanical stimulation or to lead.

It has been pointed out that there is no evidence that boxwood excites directly any form of muscle; indeed, its action appears to be depressant, since if it is painted directly upon striped or plain muscle it tends to diminish contraction. It must then produce this increased peristalsis through the nervous system, and since augmentation of intestinal movements can be obtained on the isolated and perfused intestines it follows that it is due to some action on the peripheral nervous mechanism. Perhaps the facts are best explained by supposing that in small doses it paralyses the inhibitory fibres or cells a little before the motor. Some such explanation was offered in a previous paper* to account for the purgative action of apocodeine. It may be mentioned in support of this hypothesis that these effects are only marked when the drug is injected in fairly large quantities. When it is taken by the mouth the effects on the alimentary canal (purgation) are not apparent, in which respect also it resembles the opium alkaloids. Other forms of plain muscle are also excited, and fig. 7 shows the effect on the uterus.

Effect on Intact Animals.—The effect of boxwood in small doses is much the same in all animals. Frogs exhibit first an exaggeration of reflexes followed by paralysis and cessation of respiration. If the animal at this stage be pithed the motor nerves are still active, and the heart is vigorous. An injection of 1 c.c. intravenously into a rabbit of 2 kilogrammes causes in about five minutes a well marked exaggeration of all reflexes. This is quickly followed by decided muscular weakness and rapid respiration. The weakness is shown by the squat attitude adopted by the animal, its hind limbs being thrust forward, and also by the difficulty it has in flexing the hind limbs and by the tremors. Later it loses the power of drawing up its hind limbs, and they lie extended and limp. The pupils are not altered. A quarter of an hour after injection recovery has commenced and in an hour the animal appears normal. The cat shows similar symptoms—exaggerated reflexes, great muscular weakness and respiratory trouble. The alkaloid is quite non-irritant to the eye or skin, and the nasal catarrh reported to occur in poisoning of the workers in the wood may be due either to some nervous systemic effect of the alkaloid, since animals after dosing occasionally exhibit watering from the eyes, or may be the result of some other constituent of the wood or due to the physical nature of the dust.

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* Dixon, 'Journ. Physiol.,' 1903, vol. 30, p. 97.

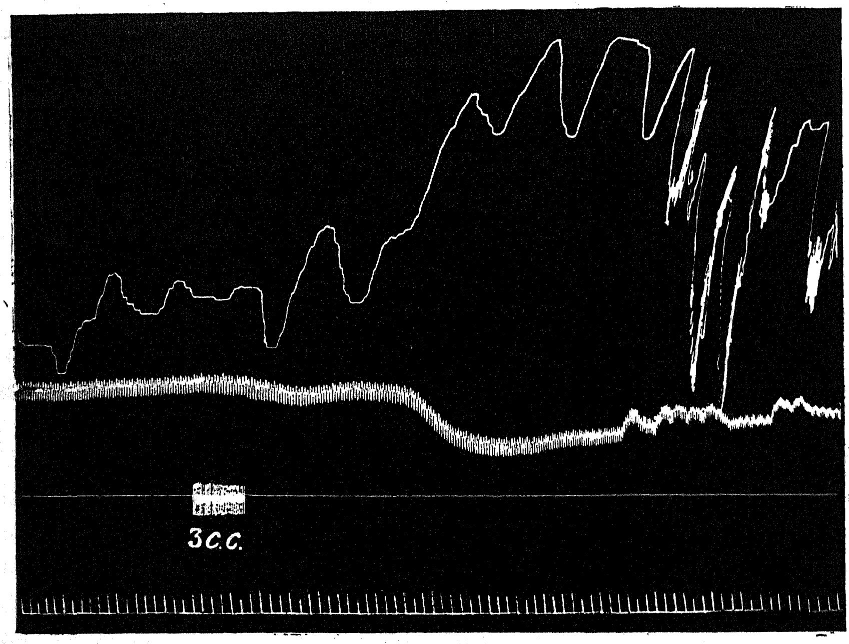

FIG. 7.—Cat. Uterine Movements. B.P. Shows increased tonus and automatic movements caused by intravenous injection of 3 c.c. of the alkaloidal solution (15 milligrammes). Time, seconds.

Discussion.—This alkaloid clearly belongs to the curare group of drugs, a group which is characterised by its members possessing three properties in common: (1) They excite the spinal cord, increase reflexes, and sometimes cause strychnine-like convulsions. (2) They paralyse nerve-cells throughout the body. (3) They paralyse motor nerve-endings.

In the case of curare the paralysis of the motor nerves overshadows its other properties, but with boxwood all the three effects are perfectly typical and are readily obtained, since they follow in the sequence given. Boxwood differs from nicotine and coniine in that there is no initial stimulant action on nerve-cells and that the motor nerve-endings are much more readily paralysed. Death from boxwood is brought about by respiratory failure, mainly central in origin, but at a time when the motor nerves are depressed and on the way to paralysis, and when the blood-pressure is low, not from cardiac depression, for the heart at this time is putting out more blood per minute than normally, but from great vaso-dilatation in the splanchnic area.

The importance of this alkaloid lies in the fact that it is alleged to cause poisoning in a percentage of the workers in the wood. Now the symptoms observed in man and put down to the action of the wood are in some respects similar to those which are found after the absorption of the drug in animals.

The sleepiness, weakness, and difficulty in respiration occur in man not constantly but only occasionally; were these symptoms constant in all cases of poisoning it would be necessary to associate them with the specific action of boxwood, but as they occur only occasionally it is more difficult to associate the action of the boxwood alkaloid after absorption with them. Moreover, by far the most important and characteristic early effect of poisoning by boxwood is the increase in reflexes; these, so far as I am aware, have never been recorded in man.

The fact that some practitioners have surmised that the symptoms may be due to cardiac failure is of little significance, since no reliable records on the activity of the heart are available, and of course to attempt to gauge the activity or output of the heart by feeling a peripheral artery or by auscultation of the chest is courting disaster.

Supposing the wood to contain 0·06 or 0·07 per cent. of alkaloid, and it being known that 8 milligrammes is sufficient to cause decided specific effects in the cat, an amount corresponding to 10 grammes of the wood, then on this basis a man should require to absorb the alkaloid from 800 grammes of wood to effect poisoning. It must be remembered, however, that whilst the amount of an alkaloid necessary to affect the heart varies in different mammals very accurately with the body weight, the same is not true for the central nervous system. For example, cocaine, a drug acting on the brain and causing convulsions, must be given to animals (frogs, rabbits, dogs, monkeys, etc.) in doses almost exactly inversely to their body weight in proportion to the relative weights of their brain substances. So that, as boxwood essentially affects the brain, it would be more accurate to compare the weight of the cat's brain with that of man in respect to their relative body weights, and then the larger the brain in proportion to body weight the less drug should be required to cause an effect. But even such an estimation would require some 200 grammes of the wood to be absorbed before symptoms of poisoning could occur. Now it will be readily admitted that the absorption of the alkaloid from such an amount of wood by workmen engaged in the operation of polishing or sawing is impracticable. Furthermore it is not possible to absorb water-soluble alkaloid from the skin in this way, and absorption is only effected with difficulty when the alkaloid is rubbed into the skin with a suitable fatty basis which will penetrate through the sebaceous and sweat glands. So that absorption when it occurs must take place through the nasobuccal mucous membrane, and it is difficult to conceive that more alkaloid than that which could be obtained from a gramme of wood at the outside could be absorbed in this fashion. The alkaloid from three times this amount of wood taken by the mouth on more than one occasion was quite without effect on a healthy man. It follows then that if it can be clearly shown clinically that poisoning definitely follows the use of boxwood in the case of certain men, and from Dr. Hay's* report this cannot be doubted, then these must possess a very extraordinary and marked idiosyncracy.

It is well known that some neurotic people, and by these I mean a class in whom a certain type of afferent stimulation produces an excessive efferent effect, are peculiarly susceptible to nerve poisons and to certain stimuli; as an illustration it is only necessary to refer to spasmodic contraction of the bronchicles from nasal irritation and to hay fever from pollen. Boxwood may claim its victims in the same way; it may be absorbed from the nasal mucous membrane and produce exaggerated local effects, increasing nasal reflexes, causing asthmatic conditions and catarrh, and the other symptoms noted. That it is possible for a drug absorbed locally to act locally we know, since strychnine injected into one temple of a man increases the field of vision on the eye of that side, and is almost without effect on the opposite eye. Some such explanation in many of the recorded cases of poisoning is the only one which I can offer as being in agreement with the facts, and it is supported by the records that only a small percentage of men working the wood have been affected, and that the commonest symptoms are those which most usually arise reflexly—headache, irritation of the eyes, nasal catarrh, and respiratory trouble, possibly asthmatic. So that although boxwood is a poison belonging to the curare group it is probably not to the general specific effects of the alkaloid after absorption that the majority of the recorded cases of poisoning must be credited.

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* Annual Report of the Chief Inspector of Factories for 1908, p. 266.

The Transformation of Proteins into Fats during the Ripening of Cheese. (Preliminary Communication.)

By M. Nierenstein.

(Communicated by Dr. Leonard Hill, F.R.S. Received November 8, 1910,—Read January 19, 1911.)

(From the Chemical Department, University of Bristol.)

It is generally accepted that the so-called ripening of cheese is accompanied by an increase in the amount of fat, and this statement is repeatedly brought forward as one of the evidences that proteins serve as a source for the formation of fat in the animal body. The production of fat during the ripening of cheese is traced to bacteria and other fungi, which break down the proteins present and set free fat, together with other degradation products.*

During some previous investigation, jointly with Dr. Breinl,† we had reason to doubt the accuracy of the view that proteins were the precursors of fat in the organism, and it appeared to me that, as the argument was partly based on a corresponding change during the ripening of cheese, this change was worth re-investigation.

The older investigators dealt either with increase of fat and decrease of casein during the ripening process, or else with the increase of fat only. Blondeau,‡ in 1869, examined Roquefort cheese, and his striking results are as follows:—

	Fresh.	1 month.	2 months.	1 year.
	Per cent.	Per cent.	Per cent.	Per cent.
Casein	85·43	61·33	43·28	40·23
Fat	1·85	16·12	18·30	18·33

But these results have so far never been confirmed.

Stohmann,§ who carefully repeated this work, and who is an authority on the chemistry of milk, concludes that "das Krasseste aber, was in diesen

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* See Leonard Hill, 'Recent Advances in Physiology and Bio-chemistry' (1908), p. 290; F. B. Leathes, 'The Fats' (1910), p. 106; and also E. Abderhalden, 'Physiologische Chemie' (1909), p. 436.

† A. Breinl and M. Nierenstein, 'Zeitschr. f. Immunitätsforschung,' 1909, vol. 2 p. 169.

‡ Blondeau, 'Ann. Chim. Physique' (4), vol. 1, p. 208.

§ Stohmann, 'Milch u. Molkereiprodukte' (1898), p. 842.

Analysen geleistet wird, besteht in der Angabe, nach welcher der aus Schafmilch bereitete Käse im frischen Zustande nur 1·85 pro cent. Fett enthalten soll. Diese als Ausgangspunkt der Vergleichung geltende Zahl ist so ungeheuerlich, dass kein weiteres Wort über dieselbe zu verlieren ist."

Nadina Sieber,* who was also unable to confirm Blondeau's experiment, concludes, "dass Blondeau Dichtung für Wahrheit gern gelten lässt," and that "die Analysen gar nicht gemacht, sondern erfunden worden sind."

Brassier,† in 1869, made a further study of this question, and showed that the ripening of cheese actually results in a decrease of both the fat and proteins. But these results were not confirmed by either Müller‡ or Kellner.§

More recent, and also more conclusive, is the work of Jacobstahl|| and of Windisch,¶ who showed that during the ripening process the percentage of the ether extract increased, but both failed to show a decrease in the proteins.

In all this work one fact stands out prominently, viz., that all the investigators evaporated down the ethereal extract from cheese and *without further investigation* weighed the residue as "fat."

Now it seemed to me more than likely that the ethereal solution might contain some other substance or substances formed during the ripening process, and that the apparent increase of fat was actually due to such products. For this reason I examined a large amount of fat extracted by ether from an old Cheddar cheese. The results I obtained showed that, besides fat, the following substances were present:—

1. Free cholesterol,
2. Cadaverine,
3. Putrescine,
4. Aminovaleric acid.

I conclude from this observation that it is not legitimate to consider that the increase in weight of the ethereal extract obtained from cheese during the ripening process affords any evidence that proteins serve as a source for fat formation in cheese, and preliminary quantitative experiments clearly showed that the actual amount of these products present with the fat was sufficient to account for the increase in the weight of the ether extract observed by so many investigators.

Any argument, then, based upon this, that proteins serve as a source for fat-formation in the animal body, falls to the ground.

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* Nadina Sieber, 'Journ. f. prakt. Chemie' (2), vol. 21, p. 203 (1880).

† Brassier, 'Ann. Chim. Physique' (4), vol. 5, p. 270.

‡ Müller, 'Landwirtsch. Jahrbücher,' vol. 1, p. 68.

§ Kellner, 'Landwirtsch. Versuchsstationen,' vol. 25, p. 39.

|| Jacobstahl, 'Pflüger's Archiv,' vol. 54, p. 484.

¶ Windisch, 'Arbeiten aus dem Kaiserlichen Gesundheitsamt,' vol. 17, p. 283.

Experimental Part.

6874 grm. of a Cheddar cheese, about four years old, were extracted with ether in a Soxhlet apparatus. The ethereal solution was evaporated to dryness, the residue dissolved in ether and precipitated with acetone, the precipitate collected, and the process repeated four times. The different ether and acetone solutions were collected and examined separately.

The precipitate (17·5 grm.), which contained traces of nitrogen, was redissolved in ether and once more precipitated with acetone, but only the precipitate formed *at once* was collected. The product thus obtained was free from nitrogen. When dissolved in warm chloroform it separated on cooling in feather-like crystalline needles, melting at 139° to 140° (cholesterol melts at 147°), and gave all reactions for cholesterol. But in order to further confirm the presence of this substance, the benzoyl derivative was prepared; this crystallised from alcohol in octahedra, which melted at 176° to 177°, and gave on analysis the following data:—

0·1527 grm. gave 0·4654 grm. CO_2 and 0·1399 grm. H_2O.
$C_{27}H_{45}O{\cdot}C_7H_5O$ requires $C = 83{\cdot}20$, $H = 10{\cdot}20$.
Found $C = 83{\cdot}12$, $H = 10{\cdot}18$.

All the acetone-ether solutions were collected and evaporated to dryness, and the residue treated in the cold with sodium carbonate and saturated with carbon dioxide. When carefully acidified with dilute sulphuric acid the filtrate gave a white precipitate which contained nitrogen. The melting point of this substance, crystallised from either benzene or alcohol and methylated spirit, was between 162° and 229°, showing that it was not pure. It was purified by dissolving 1 grm. of the material in 40 c.c. of absolute alcohol and adding 5 c.c. of boiling benzene to the solution. On cooling, well-defined needles were formed, which melted at 246° to 251°, and gave the following data on analysis:—

0·2731 grm. gave 0·5116 grm. CO_2 and 0·2334 grm. H_2O.
0·1881 „ 19·5 c.c. N_2 (19°, 758 mm.).
$C_5H_{11}O_2N$ (aminovaleric acid) requires $C = 51{\cdot}28$, $H = 9{\cdot}40$, $N = 11{\cdot}95$.
Found $C = 51{\cdot}09$, $H = 9{\cdot}36$, $N = 12{\cdot}07$.

The benzoyl derivative prepared by Fischer's method formed small prismatic needles (m. p. 173°—179°), which gave the following data on analysis:—

0·1872 grm. gave 0·4556 grm. CO_2 and 0·1139 grm. H_2O.
0·1531 „ 8·5 c.c. N_2 (16°, 756 mm.).
$C_{12}H_{15}O_3N$ requires $C = 65{\cdot}16$, $H = 6{\cdot}78$, $N = 6{\cdot}33$.
Found $C = 64{\cdot}93$, $H = 6{\cdot}81$, $N = 6{\cdot}55$.

These data point to the fact that the ethereal extract contained an acid of the aminovaleric acid type, but neither the melting point of the free acid nor that of the benzoyl derivative corresponds with any of the known acids.

As the different aminovaleric acids have lately been carefully studied by Slimmer,* in Emil Fischer's laboratory, I presume that the product I obtained was probably a mixture of two or three aminovaleric acids, especially if one considers that the melting points of both the free acid and the benzoyl derivative were not sharp.

After repeatedly washing the previously mentioned extract with sodium carbonate, the residue was freed from this salt by washing with water and then treated in the cold with a known amount of N/10 hydrochloric acid The solution obtained in this manner was then carefully neutralised with N/10 potassium hydroxide and evaporated nearly to dryness. The product was then treated with boiling alcohol and purified by means of the mercury-compound. The free bases were almost entirely soluble in alcohol, and on treating the solution with concentrated hydrochloric acid the hydrochloride of putrescine (tetramethylenediamine) crystallised out.

The chloroplatinate, picrate, and dibenzoyl derivative all agreed in their properties with the corresponding derivatives of that base. Since putrescine has been found in Emmenthal cheese,† and also, as will be shown in a further communication, in Cheddar cheese, the presence of putrescine in the cheese-fat ethereal extract may be regarded as certain.

After the putrescine hydrochloride had separated out from the alcoholic solution—a process which, according to Ackermann,‡ is quantitative—the filtrate was concentrated to about one-third of its volume, when the hydrochloride of cadaverine (pentamethylenediamine) crystallised out. The free base, the chloroplatinate, picrate, and dibenzoyl derivative were prepared, and found to correspond in all their properties with the base and its derivatives.

The presence of cadaverine in cheese has not yet been reported.

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* Slimmer, 'Ber. Deutsch. Chem. Ges.,' vol. 35, p. 400.

† Winterstein u. Thoeni, 'Zeitsch. f. physiol. Chemie,' vol. 36, p. 28; Winterstein, *ibid.*, vol. 41, p. 485. Putrescine has also been found in American Cheddar cheese (Van Slyke and E. B. Hart, 'Amer. Chem. Journ.,' vol. 29, p. 382).

‡ Ackermann, "Die Isolierung von Fäulnissbasen," 'Handbuch der Biochemischen Arbeitsmethoden (Abderhalden),' vol. 2, p. 1002.

The Action of X-Rays on the Developing Chick.

By J. F. Gaskell, M.A., M.B. Cantab., M.R.C.P.

(Communicated by W. H. Gaskell, M.D., F.R.S. Received November 11, 1910,—
Read January 19, 1911.)

(From the Pathological Laboratory, Leeds University.)

This research was undertaken at the suggestion of Prof. Grünbaum, in order to discover whether any changes were produced in the chick embryo under the action of X-rays. Embryos of various ages were examined, both directly after exposure to doses of various amounts, and also at later periods. In none were any observations obtained of changes either in the embryo as a whole or in any particular organ, with the exception of one which affected all the tissues so exposed.

It was found that X-rays had a general effect on the rate of division of the cells of the embryo, causing a diminution in the number of mitoses. This diminution was very evident in embryos exposed to long doses; for instance, chicks incubated for three days and then given a two hours' exposure, showed so marked a diminution that a considerable search was required to find any mitotic figures, while in an embryo of three days, exposed for four hours, no mitotic figures could be found.

In order to discover whether this diminution was progressive from the beginning of the exposure, or whether initially an increase of mitotic activity occurred, small doses of 5 and 10 minutes were given to embryos of four days. These were used with their controls for the purpose of making quantitative counts. The fore-brain was selected as the most suitable tissue for counting owing to the regular arrangement of the cells. That portion only was counted which was of fairly uniform thickness, and, as far as possible, median sagittal sections only were used for counting, in order to count only cells cut in their principal plane. The sections were cut 5μ thick and cells were counted by their nuclei. The results of the counts are shown in Table I. The eggs were exposed at about 20 cm. distance from the anti-cathode.

The figures obtained do not show very wide variations in each individual count, so that they should be sufficiently reliable to establish the fact that there is a progressive diminution of mitotic activity even with small doses. Counts were also attempted on the alimentary canal and the liver, but the difficulties of counting and the number of cells available did not give reliable figures; the same tendency to diminution was, however, observed.

Further evidence of a different kind supporting the view that the diminution occurs equally in all tissues will be given later in this paper.

Table I.

Expt. No.	Duration of X-ray dose.	Number of cells counted.	Mitoses observed.	Mitotic index or number per 1000.
1	Control	2,738	79	28·8
2	"	2,548	75	29·5
3	"	2,576	77	29·9
4	"	2,412	72	29·8
Total		10,274	303	29·5
1	5 minutes	3,063	72	23·5
2	"	2,592	62	23·9
3	"	2,625	62	23·6
Total		8,280	196	23·7
1	10 minutes	3,769	66	17·5
2	"	3,953	75	18·8
3	"	1,877	39	20·7
Total		9,599	180	18·7

Other specimens were incubated for three days and then X-rayed for 15 minutes, 30 minutes, and one hour respectively. The progressive diminution in mitotic activity was evident in these, though no actual counts were made. It appears, therefore, that a progressive diminution of mitotic activity takes place, under the influence of X-rays, in a rapidly growing organism such as the chick embryo.

These results are contrary to those arrived at by Cattley* in the growing root tips of plants. He found that an increasing stimulatory effect was obtained with increasing lengths of exposure up to one of 30 minutes' duration. From this point there was diminution, but not to a figure below that obtained in the controls until the exposure had lasted for two hours. His exposures were at a somewhat greater distance, 25 cm. from the anticathode, but this would not be a factor of sufficient importance to account in any way for the different type of results obtained.

It is possible that an initial stimulant effect may occur in the chick embryo also; it must, however, be very brief; smaller doses than one of five minutes' duration, in order to settle this point, have not been given in these experiments.

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* 'Journ. of Path. and Bact.,' April, 1909, vol. 13, p. 380.

The effect of this diminution of mitotic activity was then tried on the development of the embryo. Daily exposures of varying length were given to eggs which were incubated for the full 21 days. These were opened on the twenty-third day if hatching had not taken place. The results of various experiments are grouped together in Table II. In all these experiments the controls hatched out at the proper time, with the exception of three, which were apparently sterile.

Table II.

Length of daily exposure.	Distance from anti-cathode.	Results.	Average growth in days.
2 hours	15 cm.	6 eggs with no growth	0
30 minutes	12 "	2, 3rd day; 3, no growth	1·25
30 "	20 "	3, 5th day; 1, 3rd; 1, no growth	4·5
20 "	20 "	1, 10th day; 1, 7th; 1, 6th; 1, 5th; 1, 3rd	6·2
15 "	12 "	1, 14th day; 1, 13th; 1, 9th; 1 sterile	12
10 "	12 "	2 hatched; 1, 19th; 1, 17th	19·5
10 "	20 "	2 hatched; 1 opened on 17th day; 2 sterile	21
5 "	12 "	3 hatched; 1, 8th day	21

In the calculations the sterile eggs and the one in the last series which only reached the eighth day's development are neglected, this latter being decomposed.

The variations in the individual groups may be partly due to the fact that each group of eggs was exposed together in a box; the eggs were not, therefore, all equidistant from the anti-cathode. Also the X-ray tube did not give a constant dose throughout the experiments. Attempts were made to measure the dose by means of Sabaraud's pastilles, but the results were unsatisfactory, especially in the case of the short exposures.

The table shows that a daily exposure of a certain length stops further development of the embryo at a certain stage. The stage of development bears an inverse ratio to the exposure given. The critical dose, which renders the hatching of the chick doubtful, is a daily exposure of 10 minutes at 12 cm. distance in this series of experiments. Those exposed to a smaller daily dose all successfully hatched out. Those given a daily five minutes' exposure hatched out a few hours earlier than their controls but this was probably due to their shorter stay outside the incubator and their consequent shorter period for cooling. The controls were always left out 20 minutes at least, while the others only stayed out while being exposed. This is not, therefore, taken as any evidence of any stimulating effect on their growth.

The question then arose as to whether the effect was cumulative or recovery was complete. A further experiment was undertaken, and the treatment was stopped on the twelfth day. The results are shown in Table III.

Table III.

Length of daily exposure.	Distance from anticathode.	Results.	Average growth in days.
20 minutes	15 cm.	1, 5th; 1, 7th; 1, 9th; 1, 11th; 1, 13th	9
15 „	15 „	2 hatched; 1, 19th; 1, 4th; 2 sterile	20
10 „	15 „	3 hatched; 3 sterile	21
5 „	15 „	6 hatched	21

In this experiment, therefore, 20 minutes' exposure killed on or before the twelfth day, that is to say, death occurred while exposure was still going on. The average development of nine days agrees fairly well with Table II, in which a 20 minutes' dose gave development to six days. The fact that a different X-ray tube was being used may also, to some extent, account for the difference.

Those given a 15 minutes' exposure were able to hatch out, though in the previous experiments this did not occur. These embryos had not yet reached the critical stage of development at which an X-ray dose of 15 minutes takes effect; they therefore survived, and were able to hatch out at the usual time. The embryo which only reached the fourth day of development is neglected, as this was probably accidental.

Those exposed for 10 minutes and 5 minutes hatched out as in the previous experiments at the usual 21 days. The evidence from this experiment therefore tends to show that the effect is not a cumulative one, but that there is a critical point at which a certain exposure stops development.

Further experiments were intended to support this view. Eggs were to be incubated up to a certain day and then given a single exposure of length differing in the case of each group. In such a series the particular length of exposure which would just prevent further development ought to agree with that obtained from Table II, if the effect is non-cumulative. All those receiving a smaller exposure should hatch out. It has, however, at present not been possible to continue these experiments, so that this evidence is as yet lacking. Additional facts supporting the non-cumulative view have been obtained from embryos opened at various stages of development while undergoing daily exposures. If alive, they were always at the correct stage of development; none gave evidence of any retardation. The fact that the X-rayed embryos which received small doses hatched at the usual time also implies that the recovery from the X-ray effect is complete for each exposure.

The hatched chicks were kept alive to watch their further development. Unluckily, the first to hatch out under X-ray treatment did so in the late autumn, and the conditions were unfavourable for their proper development; but nevertheless no difference could be observed between them and their controls. They were killed and examined at various stages, but no differences were discovered in the development of any of their organs. This is in accord with the microscopical evidence given earlier in this paper: the X-ray action is uniform on all tissues.

Particular attention was paid to the generative organs, but neither macroscopically nor microscopically did they differ from the controls in any way. The chicks obtained from the experiment in Table III are at present being brought up with the intention of testing their breeding capabilities, to see if any action has occurred on their generative cells. They are at present all normal in appearance.

The meaning of these experiments, and the probable action of X-rays on growing tissues, may now be considered. It has been shown by Minot,* by actually counting the number of mitoses per 1000 cells in the various tissues of embryos of different ages, that throughout embryonic life a rapid diminution of mitotic activity is going on. He calls the figures obtained the mitotic index for that particular tissue.

This mitotic index varies at any particular stage of development according to the rapidity of growth of the particular organ at that moment, but this variation is not sufficient to obscure the general diminution of the index which is taking place. The effect of X-rays is to bring about rapidly a progressive lowering of this mitotic index in all the tissues of the developing embryo. This lowering is not permanent, and is completely recovered from so long as it has not been of too great a degree. If, however, the exposure has been of a certain critical length, recovery no longer takes place, and the embryo dies. This critical dose depends upon the stage of development reached by the embryo, that is to say, upon the value of the general mitotic index at that stage; the larger the mitotic index is—that is to say, the greater the reproductive activity—the larger is the X-ray dose required to prevent further development.

The X-rays are, therefore, directly antagonistic to the reproductive activity of the cell, and act in such a way as to lower, and finally to destroy, its power of reproduction.

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* "The Problem of Age, Growth, and Death," 'Popular Science Monthly,' 1907, vol. 71, p. 510.

I am greatly indebted to Prof. Grünbaum for the suggestion of this research, for the use of the apparatus, and for his advice and encouragement. For some months previously he had been working at this subject, and had been assisted by a grant from the Grant Committee of the Royal Society. I am fully aware that this paper is of the nature of a preliminary communication, and that the investigation is by no means complete. As, however, I am unlikely to be able to continue it at present, I have thought it best (with the concurrence of Prof. Grünbaum) to publish it in its present unfinished condition rather than to wait for a further opportunity to complete the investigation.

Summary.

1. The action of X-rays is to lower the mitotic activity of growing tissues.
2. If this diminution is not too great, complete recovery occurs.
3. If the diminution is above a certain degree, recovery does not take place.
4. The critical dose, which just prevents recovery, varies with the stage of development of the embryo, decreasing as the mitotic index decreases.
5. No other macroscopic or microscopic changes have been observed to take place under the action of X-rays, either in the proportions or structure of the various tissues.

Experiments to Ascertain if Antelope may Act as a Reservoir of the Virus of Sleeping Sickness (Trypanosoma gambiense).

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Introduction.

The question of a reservoir of the virus of Sleeping Sickness, other than man and his domestic animals, is of the utmost importance.

Now man and the domestic animals have been removed from the Lake-shore of the mainland for some two and a half years, and from the islands since September, 1909. The effect of this depopulation has been to make a two mile area along the northern shores of the Lake virtually a game reserve, in which water-buck, bush-buck, reed-buck, Speke's *Tragelaphus*, hippopotami, wild pig, and other large game abound. The game water freely at the Lake-shore, and small herds of antelope may frequently be seen grazing on the grassy hillsides overlooking the Lake.

Notwithstanding the removal of man and his domestic animals, the Lake-shore *Glossina palpalis* continued to infect susceptible animals with Sleeping Sickness up to the end of March, 1910. The Commission, therefore, endeavoured to find answers to the following questions:—

1. Can antelope be infected with Sleeping Sickness by the bites of laboratory-bred and laboratory-infected *Glossina palpalis*?

2. If antelope can be infected with the virus of Sleeping Sickness, can they transmit the infection to laboratory-bred *Glossina palpalis* when these flies are allowed to feed upon them? Further, if these *Glossina palpalis* become infected, can they transmit the virus to susceptible animals?

3. If these *Glossina palpalis* become infected with the virus of Sleeping Sickness, what percentage are so infected?

4. How does Sleeping Sickness affect the health of the antelope?

5. Lastly, are antelope living in the fly-area naturally infected with Sleeping Sickness?

Should all these questions be answered in the affirmative, the importance of the observation is patent. The continued infectivity of the flies on the Lake-shore would be explained. Whilst the movements of man and the domestic animals can, to some extent, be controlled by administrative measures, the movements of antelope in search of new grazing grounds would be almost impossible to check, owing to the difficult nature of the forested and elephant-grass country which in so many parts borders the Uganda shores of the Lake. Game laws would require modification, and the destruction of the game in the neighbourhood of the lakes and *palpalis*-frequented rivers would become a factor in the control of Sleeping Sickness.

To answer the above queries the following experiments were devised and carried out:—

1. *Can Antelope be Infected with Sleeping Sickness by the Bites of Laboratory-bred and Laboratory-infected Glossina palpalis?*

Eleven buck in all were employed in this experiment. Four bush-buck (*Tragelaphus sylvatica?*), six reed-buck (*Cervicapra arundinacea*), and one water-buck (*Cobus defassa*). Other buck, such as "oribi" (*Cephalophus grimmi*) and "entalaganya" (*Cephalophus equatorialis*), were obtained by the Commission, but did not survive long in captivity.

As each buck arrived its blood was usually injected, subcutaneously, into monkeys or rats, to ascertain if the blood was naturally infected with trypanosomes. The method then adopted in each case to infect the antelope with Sleeping Sickness was as follows: A cage of clean* laboratory-bred *Glossina palpalis* was fed on a monkey known to be infected with a *human strain* of *Trypanosoma gambiense.* This cage of artificially-infected flies was then fed on healthy animals until one of the animals (monkeys) became infected with Sleeping Sickness. Having proved that the flies were infective, the cage of flies was then fed, about five minutes daily, for several days, on one or other of the buck. The buck's blood was then examined daily for trypanosomes, and was further tested for Sleeping Sickness by its inoculation into monkeys or rats.

The experiments follow in full detail.

Experiment 2328. Bush-buck.

This bush-buck was fed on for five days (March 24, 25, 26, 28, and 29, 1910) by laboratory-bred *Glossina palpalis* which were known to be infected with a *human strain* of *Trypanosoma gambiense.*

On April 8, 15 days after the first feed of the infected flies on this buck, 5 c.c. of the buck's blood were injected, subcutaneously, into a healthy monkey.

April 19, monkey showed *Trypanosoma gambiense* in its blood. The trypanosomes were verified by examination in fresh and stained preparations of the blood.

Result.—Positive.

Remarks.—The preliminary inoculation of the blood into susceptible animals, to ascertain if the buck's blood naturally harboured trypanosomes, was not made in this case. Though examined almost daily from March 29 to August 5, 1910, *Trypanosoma gambiense* was never seen in the blood of this antelope. Fresh and stained blood films were made and examined. On one occasion only—April 20—*Trypanosoma ingens* was seen in a fresh preparation. Monkeys are not inoculable with this parasite.

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* The word "clean," applied here and throughout this paper to laboratory-bred *Glossina palpalis*, means flies which have newly hatched out from pupæ in the laboratory and have never fed.

Experiment 2357. Reed-buck.

April 8, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey, to ascertain if buck's blood naturally harboured trypanosomes. This monkey's blood was examined bi-weekly for a month. Monkey remained healthy, no trypanosomes appearing in its blood.

Buck was fed on for 12 days, between April 7 and 20, 1910, by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense*. On the 13th day after the first feed of these flies, 3 c.c. of the buck's blood were inoculated into a normal monkey. Examined bi-weekly for a month this monkey remained healthy.

Trypanosoma gambiense having appeared in the buck's blood on May 2, a further inoculation of 5 c.c. of its blood was made into a normal monkey on this date. This monkey showed *Trypanosoma gambiense* in its blood on May 10.

Result.—Positive.

Remarks.—This reed-buck was evidently free from any trypanosome infection inoculable into monkeys on its arrival at the laboratory. *Trypanosoma gambiense* appeared for the first time in the buck's blood in scanty numbers on May 2, 1910, 25 days after the first feed of the supposed infective *Glossina palpalis*. On May 3 and 4, the trypanosomes were fairly numerous in the blood, and on the 5th and 6th, scanty numbers only were seen. After May 6, *Trypanosoma gambiense* was never again seen in the blood, though examined for almost daily up to August 5, 1910. The long period of 25 days which elapsed between the first feed of the supposed infected *Glossina* and the appearance of the trypanosomes in the buck's blood may be accounted for by the supposition that the infected fly or flies in the cage which was fed on the buck from April 7 to April 19 had died before they had fed on the buck. On April 20, a fresh cage of *Glossina palpalis*, known to be infected with a *human strain* of *Trypanosoma gambiense* was fed once only, on this buck, and on the 12th day after this feed, on May 2, trypanosomes appeared in the buck's blood. This supposition is probably correct, and further accounts for the failure of the inoculation on April 20.

Experiment 2359. Reed-buck.

On April 8, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey, to ascertain if buck's blood naturally harboured trypanosomes. This monkey's blood was examined bi-weekly for a month. Monkey remained healthy.

Buck was fed on for six days (April 25—30, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense*.

On May 6, the 11th day after flies' first feed, *Trypanosoma gambiense* appeared in buck's blood in fair numbers, and 5 minims were injected, subcutaneously, into normal white rat. On May 11 this rat showed *Trypanosoma gambiense* in its blood.

Result.—Positive.

Remarks.—This reed-buck was evidently free from any trypanosome infection inoculable into monkeys on its arrival at the laboratory. *Trypanosoma gambiense* appeared for the first time in its blood in fair numbers on May 6, 1910, again on 7th, not examined for on 8th, scanty on 9th, absent on 10th, scanty on 11th, very numerous on 12th, and never seen again, though examined for almost daily, till August 5.

Experiment 2371. Bush-buck.

On April 13, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey, to ascertain if buck's blood naturally harboured trypanosomes. This monkey's blood was examined bi-weekly for a month. Monkey remained healthy.

Buck was fed on for eight days (April 22 and 23, and from April 25 to 30, 1910, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense*.

On May 4, the 12th day after the infected flies' first feed on the buck, *Trypanosoma gambiense* appeared in the buck's blood in fair numbers.

On May 5, trypanosomes were numerous, and 2 c.c. of the buck's blood were injected, subcutaneously, into a normal monkey. On May 13 this monkey showed *Trypanosoma gambiense* in its blood.

Result.—Positive.

Remarks.—Buck was free from trypanosomes inoculable into a monkey on its arrival at the laboratory. *Trypanosoma gambiense* appeared in buck's blood for the first time, in scanty numbers, on May 3, 1910, and again in fair numbers on May 4; thereafter no trypanosomes were seen up to August 5, 1910, though the blood was almost daily examined.

Experiment 2378. Water-buck.

On April 13, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey, to ascertain if the buck naturally harboured trypanosomes in its blood. This monkey was examined bi-weekly for one month; monkey remained healthy.

Buck was fed on for eight days (April 22, 23, 25 to 30, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense*. *Trypanosoma gambiense* was never seen in this buck's blood, though examined for almost daily from April 22 to August 5, 1910.

On May 5, 5 c.c. of the buck's blood were injected, subcutaneously, into a normal monkey, an interval of 13 days having elapsed since first feed of the infected flies. This monkey showed *Trypanosoma gambiense* in its blood on May 13, 1910.

Result.—Positive.

Remarks.—Buck was free from trypanosomes inoculable into a monkey on its arrival at the laboratory. Though *Trypanosoma gambiense* never appeared in the buck's blood, yet a positive result was obtained on its inoculation into a monkey, and, as will be seen below (Table II), clean laboratory-fed flies fed on this buck for several days became infected with *Trypanosoma gambiense*.

Experiment 2427. Reed-buck.

On May 4, 1910, 1 c.c. of this buck's blood was injected, subcutaneously, into a normal white rat. This rat, examined bi-weekly, for one month, remained healthy.

Buck was fed on for six days (May 2 to 7, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense*.

On May 9, the seventh day after infected flies' first feed on the buck, *Trypanosoma gambiense* appeared in scanty numbers in its blood for the first time. One cubic centimetre of the blood was then injected, subcutaneously, into a normal white rat. On May 16, *Trypanosoma gambiense* appeared in this rat's blood.

Result.—Positive.

Remarks.—On its arrival at the laboratory the buck was free from trypanosomes inoculable into a rat. A few *Trypanosoma gambiense* appeared for the first time on May 9, 1910, in buck's blood; they were present in fair numbers on the 10th, scanty again on the 11th, and were seen for the last time, in fair numbers, on May 12. Almost daily blood examinations were made, with negative results, up to August 5, 1910.

Experiment 2428. Bush-buck.

On May 4, 1910, 1 c.c. of this buck's blood was injected, subcutaneously, into a normal white rat. This rat remained healthy, its blood being examined for one month after the injection.

Buck was fed on for 13 days (May 2—16, inclusive, May 9 and 15 being excepted) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense*. *Trypanosoma gambiense* was never seen in this animal's blood, though examined for almost daily from May 4 to August 5, 1910.

On May 16, 14 days after infected flies' first feed on the buck, a few drops of the buck's blood were injected, subcutaneously, into a normal white rat. This rat showed *Trypanosoma gambiense* in its blood on May 23. No trypanosomes were ever seen in buck's blood.

Result.—Positive.

Remarks.—On its arrival at the laboratory the buck was free from trypanosomes inoculable into a rat. No trypanosomes were ever seen in the buck's blood, nevertheless its blood was infective on inoculation, and, as will be seen later (Table II), capable of infecting clean laboratory-bred *Glossina palpalis.*

Experiment 2429. Reed-buck.

On April 13, 1910, 5 c.c. of this buck's blood were injected, subcutaneously, into a normal monkey. This monkey remained healthy, its blood being examined bi-weekly for a month after the injection. On May 3, 1910, 1 c.c. of the buck's blood was injected, subcutaneously, into a normal rat. This rat remained healthy, its blood also being examined bi-weekly for a month after the injection.

The buck was fed on for eight days (May 2—7, and 9 and 10, inclusive) by *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense.*

On May 11, the ninth day after the infected flies' first feed on the buck, *Trypanosoma gambiense* appeared in scanty numbers in its blood for the first time. On May 13, the trypanosomes being numerous in the blood of the buck, a few drops of the blood were injected, subcutaneously, into a normal white rat. This rat showed *Trypanosoma gambiense* in its blood on May 17.

On July 2, 1910, this buck accidentally broke its leg and had to be killed, 4 c.c. of its heart's blood being injected into a normal monkey. This monkey showed *Trypanosoma gambiense* in its blood on July 12.

Result.—Positive.

Remarks.—On its arrival at the laboratory the buck was free from trypanosomes inoculable into monkeys or rats. *Trypanosoma gambiense* appeared in its blood for three days —on May 11 for the first time in scanty numbers, on May 12, many, and on May 13, very many—thereafter no trypanosomes were seen, though almost daily examinations were made up to July 2, 1910. It will be noted that the buck's blood was still infected with *Trypanosoma gambiense* on July 2—that is, 50 days after the last date (May 13) that trypanosomes were seen in it.

Experiment 2431. Reed-buck.

On May 3, 1910, 1 c.c. of this buck's blood was injected, subcutaneously, into a normal white rat. Rat remained healthy, its blood being examined bi-weekly for a month after the injection.

The buck was fed on for six days (May 2—7, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense.*

On May 12, 1910, the tenth day after the infected flies' first feed, *Trypanosoma gambiense*, in scanty numbers, appeared for the first time in the blood of the buck. On May 13, the trypanosomes being numerous in the blood of the buck, a few drops of its blood were injected, subcutaneously, into a normal white rat. This rat showed numerous *Trypanosoma gambiense* in its blood on May 20.

Result.—Positive.

Remarks.—On its arrival at the laboratory the buck was free from trypanosomes inoculable into rats. *Trypanosoma gambiense* appeared in the buck's blood, in scanty numbers, for the first time on May 12, and were present in large numbers on May 13 and 14.

We have now to record one of the most important and suggestive observations in this series of experiments. *From May* 14 *to June* 27 *the blood was examined almost daily, and on the latter date—June* 27—44 *days after trypanosomes were last seen,* Trypanosoma gambiense *reappeared for one day in this buck's blood in fair numbers.* Their identity was established by careful examination in wet and stained preparations of the blood.

(N.B.—Two mechanical transmission experiments were carried out with this buck. In the first, 50 flies were used and were fed on the buck for the three days the trypanosomes were seen in its blood. *Result.*—Negative. In the second, 100 flies were used, and were fed for four days on the buck. No trypanosomes were seen in the buck's blood during these days. *Result.*—Negative. In the first experiment four hours, and in the second one hour, elapsed between the feed on the buck and the feed on the healthy monkey.)

Experiment 2445. Reed-buck.

No preliminary inoculation of this buck's blood was made on its arrival at the laboratory.

The buck was fed on for seven days (May 6 and 7, and 9—13, inclusive) by laboratory-bred *Glossina palpalis* known to be infected with a *human strain* of *Trypanosoma gambiense.*

On May 14, 1910, the eighth day after the infected flies' first feed, *Trypanosoma gambiense* appeared in the blood of the buck for the first time. On May 20 the buck died, and a small quantity of its heart's blood was injected, subcutaneously, into a normal white rat. On June 2 rat showed *Trypanosoma gambiense* in its blood. (This rat was overlooked from May 24 to June 2, and its blood was not examined between these dates.)

Result.—Positive.

Remarks.—The blood of this buck was (with the exception of May 8) examined daily from May 6 to 13 with negative results for trypanosomes. On May 14, *Trypanosoma gambiense* appeared for the first time in its blood in scanty numbers; on the 16th, 17th, and 18th they were very numerous; on the 19th they were again scanty, and on the day of death, May 20, 1910, no trypanosomes could be found, the peripheral and heart's blood being examined.

Table I gives results of feeding infected *Glossina palpalis* on healthy antelope.

Table I.

No. of experiment.	Species of antelope.	No. of days infected flies fed.	No. of days before trypanosomes appeared.	Result.		Remarks.
				Positive.	Negative.	
2328	Bush-buck	5	—	+		Trypanosomes never seen.
2357	Reed-buck	12	25	+		
2359	"	6	11	+		
2371	Bush-buck	8	12	+		
2372	"	6	8	+		
2378	Water-buck	8	—	+		Trypanosomes never seen.
2427	Reed-buck	6	7	+		
2428	Bush-buck	13	—	+		Trypanosomes never seen.
2429	Reed-buck	8	9	+		
2431	"	6	10	+		
2445	"	7	8	+		

From these experiments it is shown that antelope may be readily infected with Sleeping Sickness by the bites of artificially-infected tsetse flies. Eleven antelope were used, and in every case a positive result was obtained. It will be remembered that in similar experiments made with cattle the same result was obtained.

2. *If Antelope can be Infected with the Virus of Sleeping Sickness, can they Transmit the Infection to Clean Laboratory-bred Glossina palpalis when these Tsetse Flies are allowed to Feed upon Them? Further, if these Glossina palpalis become infected, can they transmit the Virus to Susceptible Animals?*

It has now been proved that water-buck, reed-buck, and bush-buck can be infected with the virus of Sleeping Sickness, with what would seem to be unfailing regularity. Should, however, these antelope be incapable of infecting the *Glossina palpalis* with *Trypanosoma gambiense*, the fact is of academic importance only. On the other hand, should the results of feeding clean laboratory-bred *Glossina palpalis* on these infected buck give positive results when these flies are subsequently fed on normal susceptible animals, a further step has been made in the search for a reservoir of the virus of Sleeping Sickness, other than man and his domestic animals.

The method adopted to test this second query was carried out as follows :—Clean, laboratory-bred *Glossina palpalis* were fed for several days on an infected buck. After an interval of starvation of 24 hours or more the flies were transferred to healthy animals and fed daily. When the healthy animal showed *Trypanosoma gambiense* in its blood the experiment was stopped, and the surviving flies were dissected as soon as possible.

The result of 24 experiments carried out on these lines is given in the following table:—

Table II.—Giving the Result of Feeding Laboratory-Bred Flies on Antelope Infected with Sleeping Sickness.

No. of experiment.	No. of clean flies used.	Species of antelope flies fed on.	No. of days flies fed on antelope.	No. of days before flies became infective.	Result.		Remarks.
					Positive.	Negative.	
2346	160	Bush-buck 2328	12	29	+		Buck 2328 never showed *T. gambiense* in blood. In spite of this, flies fed on it became infected 55 days after the buck's infection.
2384	100	"	8	28	+		
2414	70	"	6	29	+		
2501	100	"	8	39	+		
2351	100	Reed-buck 2357	7	41	+		Buck 2357 showed *T. gambiense* in its blood for 5 days only.
2500	100	"	8	—		—	
2510	100	"	5	—		—	
2507	200	Reed-buck 2359	6	44	+		Buck 2359 showed *T. gambiense* in its blood for 7 days only.
2421	50	Bush-buck 2371	6	—		—	Buck 2371 showed *T. gambiense* in its blood for 3 days only.
2477	60	"	6	29	+		
2499	100	Bush-buck 2372	8	—		—	Buck 2372 showed *T. gambiense* in its blood for 2 days only.
2451	95	Water-buck 2378	6	30	+		Buck 2378 never showed *T. gambiense* in its blood.
2478	60	"	6	—		—	
2559	50	"	4	—		—	
2454	110	Reed-buck 2427	6	24	+		Buck 2427 showed *T. gambiense* in its blood for 4 days only.
2456	60	"	4	33	+		
2508	100	"	6	30	+		
2485	50	Bush-buck 2428	7	28	+		Buck 2428 never showed *T. gambiense* in its blood.
2460	50	Reed-buck 2429	4	27	+		Buck 2429 showed *T. gambiense* in its blood for 3 days only.
2543	100	"	6	49	+		
2464	55	Reed-buck 2431	3	28	+		Buck 2431 showed *T. gambiense* in its blood for 4 days only. In spite of this, flies fed on it became infected 81 days after its infection.
2544	90	"	6	36	+		
2592	100	"	5	43	+		
2476	50	Reed-buck 2445	4	—		—	Buck 2445 showed *T. gambiense* in its blood for 6 days only.

On glancing at Table II it will be seen that 24 experiments in all were carried out. Of these, 17 were positive (70·84 per cent.), and seven negative (29·16 per cent.). The shortest time which elapsed before the flies became infective was 24 days, the longest 49 days, and the average 33·35 days.

Compare these results with those detailed in the 'Proceedings' of the Royal Society, B, 1910, vol. 82, p. 374, Table III. Of the 42 experiments there described, only 8 (19 per cent.) were positive. The clean laboratory-bred flies were fed on *Trypanosoma gambiense*-infected monkeys in 36 of those experiments, in one case on a Sleeping Sickness patient, and in five cases on oxen infected with the virus of Sleeping Sickness.

Positive results were obtained from all the buck on at least one occasion, with the exception of bush-buck, Experiment 2372, and reed-buck, Experiment 2445. Only two experiments were carried out from these buck, one from each, viz., Experiments 2499 and 2476. In Experiment 2499 the flies were fed on the antelope 19 days after the trypanosomes had disappeared from its blood, as far as microscopical examination went. In Experiment 2476 the flies were non-infective to monkeys up to the 45th day after their first feed on the infected buck. This latter experiment was proceeding when the Commission left Uganda, and a positive result may yet have to be recorded.

The most significant of the above observations is the one in which it is shown that 55 days after the last feed of infected *Glossina palpalis* on bush-buck, Experiment 2328, the blood of this buck was capable of infecting clean laboratory-bred flies, though *Trypanosoma gambiense* had never been seen in its blood.

To illustrate how these experiments were carried out, full details of two are given. They are typical of the methods adopted. One positive and one negative experiment have been chosen. (See next page.)

These experiments show that antelope of the water-buck, reed-buck, and bush-buck species, when infected with the virus of Sleeping Sickness, can transmit the infection to clean laboratory-bred *Glossina palpalis*. The infected antelope's blood was, in one case, infective to *Glossina palpalis* for at least 81 days, and in another for at least 55 days. These experiments further show that the flies, when infected by the virus of Sleeping Sickness obtained from the blood of infected antelope, are capable of transmitting the virus to susceptible animals.

Experiment 2501.—To ascertain if Laboratory-Bred *Glossina palpalis* become Infective when Fed on Antelope whose Blood contains *Trypanosoma gambiense.*

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910.				
May 23—28 ...	1—5	Flies fed 5 minutes daily on Bush-buck 2328.		100 flies used.
„ 29	6	Flies starved.		
„ 30—31 ...	7—8	Flies again fed 5 minutes daily on Buck 2328.		
June 1............	9	Flies starved.		
„ 2—July 7	10—45	Flies fed 5 minutes daily on normal Monkey 2517.		July 8, Monkey 2517 shows *T. gambiense* in its blood to-day. Allowing 7 days for incubation of *T. gambiense* in monkey's blood, then the *G. palpalis* became infective on the 39th day after their first infected feed on Buck 2328.
July 8	46	Flies starved, as Monkey 2517 shows *T. gambiense* in its blood.	+	
„ 9	47	The 57 surviving *G. palpalis* dissected; 20·3 per cent. of these flies showed heavy intestinal infection with flagellates (*T. gambiense*).		

Remarks.—This is the experiment referred to above, where the blood of a buck was capable of infecting clean laboratory-bred flies 55 days after last feed of infected flies on the buck.

Experiment 2499.—To ascertain if Laboratory-Bred *Glossina palpalis* become Infective when Fed on Antelope whose Blood contains *Trypanosoma gambiense.*

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910.				
May 23—28	1—5	Flies fed 5 minutes daily on Bush-buck 2372.		100 flies used.
„ 29	6	Flies starved.		
„ 30—31	7—8	Flies again fed 5 minutes daily on Buck 2372.		
June 1	9	Flies starved.		Monkey 2552 was examined bi-weekly from June 13 to August 12. It remained healthy. The surviving *G. palpalis* were not dissected as experiment was negative.
„ 2—11	10—19	Flies fed on Cock 2518.		
„ 12	20	Flies starved.		
„ 13—July 12	21—50	Flies fed 5 minutes daily on clean Monkey 2552.	–	
July 13 and following days.	51	Flies allowed to die. Not dissected as experiment negative.		

3. *If Glossina palpalis can be infected with the Virus of Sleeping Sickness by Feeding on the Blood of Trypanosoma gambiense-infected Antelope, what Percentage are found to be so Infected?*

It has been shown now that antelope can be infected with the virus of Sleeping Sickness, that when so infected they can infect the fly, and the fly in its turn can convey the disease to susceptible animals.

These facts form a serious sequence of events, which constitute a danger not formerly appreciated in the administrative measures adopted to check the spread of the disease. What is the extent of the danger? A part of this large and important query can be answered if one can give an idea of the percentage of *Glossina palpalis* that become infected with the virus of Sleeping Sickness after they have fed on the infected antelope.

Throughout all these experiments only clean laboratory-bred flies were employed. The fact that there is no hereditary transmission of trypanosomes in *Glossina palpalis* is considered to have been so conclusively proved that two of the members of the Commission have allowed several hundreds of clean laboratory-bred flies to bite them. Further, no evidence has ever been obtained by the Commission that these flies became infected with any flagellate by contact with other flies or fouled cages. Thus, any flagellates found in the laboratory-bred *Glossina palpalis* in these experiments must be considered to be derived from the infected antelope.

In some of the experiments the flies were fed, for varying periods, upon fowls. As will be shown in a further paper, the Commission found an avian trypanosome in some of the fowls obtained for experimental purposes. It would, therefore, be a fair criticism to state that a percentage of the flagellates found on dissection of the *Glossina* were avian in origin, were it not for the fact that negative experiments went to prove that this fowl trypanosome did not develop in the *Glossina*. It is also true that on one occasion the Commission thought they had succeeded in infecting a fowl with *Trypanosoma gambiense*; it may, therefore, be argued that the fowls fed upon in some of these experiments were naturally infected with *Trypanosoma gambiense*, and that the *Glossina* obtained their infection from such naturally-infected fowls and not from the antelope. Though many experiments were devised and carried out to try and confirm this one positive result, all efforts to infect fowls with Sleeping Sickness were so uniformly negative that the Commission must consider the one "positive" result to be an error.

A reference to Table III will show that *Glossina palpalis* were infected by antelope blood where no fowls were ever fed on; in fact, it will be noticed

Table III.—Giving the Percentage of Flies which became Infected when Fed on Infected Antelope. Flies fed at first on the infected antelope and afterwards only on healthy monkeys.

No. of experiment.	Species of antelope flies fed on.	No. of flies used.	No. of flies dissected.	No. of infected flies found.	Percentage of infected flies.	Remarks.
2346	Bush-buck 2328	160	122	21	17·2	The gut-contents of 6 infected flies injected into a rat gave it Sleeping Sickness.
2384	„ 2328	100	91	10	11·0	No injection of infected flies.
2414	„ 2328	70	70	9	12·8	Five infected flies injected into a rat gave it Sleeping Sickness.
2501	„ 2328	100	57	12	21·0	No injection of infected flies.
2351	Reed-buck 2357	100	84	9	10·7	„ „ „
2507	„ 2359	200	80	2	2·5	„ „ „
2477	Bush-buck 2371	60	47	4	8·5	„ „ „
2478	Water-buck 2378	60	—	—	—	Negative experiment. Flies not dissected.
2559	„ 2378	60	—	—	—	„ „ „
2454	Reed-buck 2427	110	92	13	14·2	One infected fly injected into a rat gave it Sleeping Sickness.
2485	Bush-buck 2428	50	26	3	11·5	No injection of infected flies.
2460	Reed-buck 2429	50	38	5	13·1	„ „ „
2543	„ 2429	100	—	—	—	Negative experiment. Flies not dissected.
2544	„ 2431	90	53	5	16·6	No injection of infected flies.
2592	„ 2431	100	78	1	1·3	„ „ „
2476	„ 2445	50	—	—	—	Negative experiment. Flies not dissected.

Remarks.—Of these 16 experiments, 12 were positive and 4 negative. In the positive experiments, 838 flies were dissected. Of these, 94 showed heavy intestinal infection with developmental forms of *Trypanosoma gambiense*, viz.,' 11·2 per cent. Of these infected flies, 43 were males and 51 females. If to these 838 flies be added all the *Glossina palpalis* of the negative experiments, we get a total of 1108 flies, and a percentage of 8·5 infected. In only 3 of the positive experiments was an injection made into susceptible animals of the citrated gut-contents of the infected flies. All three gave positive results.

that a higher percentage of negative results were obtained where fowls were introduced into the experiments. In some of the experiments the flies were dissected as they died throughout the whole experiment, and in others the flies were dissected only when the experiment became positive. If the experiment was a negative one the flies were not dissected. In order to avoid over estimations of the percentage of infected flies, no fly was called infected unless its gut was *swarming* with trypanosomes, and all the flies dissected during the earlier days of the experiments have been included.

No fly was found to be infected with trypanosomes before the 19th day after its first infected feed on a buck. The tables otherwise explain themselves.

The methods of procedure adopted in the experiments detailed in the following table were precisely the same as those of Table III, with this one exception: The flies were, for varying periods, fed upon fowls as well as upon monkeys.

Table IV.—Giving the Percentage of Flies which became Infected when Fed on Infected Antelope. Flies fed at first on the infected antelope and afterwards on fowls, before being fed on healthy monkeys.

No. of experiment.	Species of antelope flies fed on.	No. of flies used.	No. of flies dissected.	No. of infected flies found.	Percentage of infected flies.	Remarks.
2500	Reed-buck 2357	100	—	—	—	Negative experiment. Flies not dissected.
2510	,, 2357	100	—	—	—	,, ,,
2421	Bush-buck 2371	50	—	—	—	,, ,,
2499	,, 2372	100	—	—	—	,, ,,
2451	Water-buck 2378	95	93	2	2·1	No injection of infected flies.
2456	Reed-buck 2427	60	50	9	18·0	,, ,,
2508	,, 2427	100	68	6	8·8	Three infected flies injected into a rat gave it Sleeping Sickness.
2464	,, 2431	55	53	8	15·0	No injection of infected flies.

Remarks.—It will be seen that when the flies were fed on fowls and monkeys in these eight experiments, four were positive and four negative. In the positive experiments 264 flies survived for dissection: of these 25 showed heavy intestinal infection with developmental forms of *Trypanosoma gambiense*, *i.e.* 9·47 per cent. Of the infected flies 11 were males and 14 females. If to these 264 flies be added all the *Glossina palpalis* of the four negative experiments, we get a total of 614 flies and a percentage of 4·0 infected. In the only experiment where an injection was made into a susceptible animal of the pooled citrated gut-contents of infected flies, the result was positive.

An analysis of Tables III and IV brings out the following interesting points:—

In Experiment 2501, Table III, it is seen that 21 per cent. of the *Glossina palpalis* were infected, out of the 57 flies that survived for dissection on the 47th day of the experiment. These flies were infected by bush-buck, Experiment 2328, which had never shown *Trypanosoma gambiense* in its blood; and 55 days had elapsed since any infected *Glossina* had fed on this buck.

Sixteen out of the twenty-four experiments were positive. If all the *Glossina palpalis* dissected' in these positive experiments be grouped together, it is seen that a total of 1,102 flies were examined. Of these, 119 flies—54 male and 65 female—were infected with developmental forms of *Trypanosoma gambiense*—that is, 10·8 flies in every hundred became infected, the sexes being about equally implicated. The highest percentage of infected flies in any one of the positive experiments was 21 per cent., in Experiment 2501, and the lowest was 1·3 per cent., in Experiment 2592. If to the total of 1,102 flies dissected in the positive experiments be added *all* the *Glossina* used in *all* the negative experiments, we get a total of 1,722 flies employed, and 6·9 flies in every hundred infected.

It is perhaps worth noting that a diet for the fly of antelope, fowl, and monkey blood gave a higher percentage of *negative* results and a lower percentage of flies infected than a diet of antelope and monkey blood only. This was quite unexpected, for the Commission, as a result of many experiments and considerable experience, were of the opinion that fowl's blood assisted the development of *Trypanosoma gambiense* in *Glossina palpalis.*

4. *How does Sleeping Sickness Affect the Health of Antelope?*

This point is of considerable importance. If the disease killed the antelope within a short time of infection, or even if it seriously affected their health so as to render them incapable or unwilling to move about freely, the facts detailed above would lose some part of their practical value.

The word "health" is not used here in a technical sense—that is to say, the health was not estimated by a series of blood counts and temperature charts. Interesting as such observations would have been, the Commission regret they were too short-handed and too much pressed by other work to carry them out.

Careful observations were made daily to answer the following questions:—Did the infected antelope during the time they were under observation appear sick? Did they become emaciated? Was there loss of health and strength? Were there corneal opacities, œdematous swellings, conjunctival discharges, or staring coats? These questions may at once be answered in the negative, except in the case of Reed-buck 2445, which will be referred to later below.

When the antelope were brought to the laboratory by the native hunters they invariably suffered from exhaustion, due probably to a combination of causes, such as fright, confinement for two to four days in cages too small to allow free movement, insufficient water and food, and the rough usage undergone when being caught. As a result of these unfavourable conditions, each antelope was kept under observation for a week or two before any experiments were undertaken. During this time they were well fed and comfortably housed in reed kraals erected in a fly-proof house. Some of the antelope, especially the "oribi" and "entalaganya," died during the first fortnight. The 11 survivors were the subjects of these experiments.

Nine of these buck were kept under daily observation for four months after becoming infected with *Trypanosoma gambiense.* They remained apparently in perfect health.

The remaining two antelope were reed-buck. One, Experiment 2429, lived, and appeared very healthy, for 93 days after its infection. It then accidentally broke its leg and had to be killed. A *post-mortem* examination was made, and no evidence of trypanosomiasis was found. The other buck, Experiment 2445, arrived at the laboratory in a poor state of health, and died 12 days after its infection. There was no sign of trypanosomiasis at the *post-mortem* examination.

It is therefore evident that antelope infected with the virus of Sleeping Sickness may live in apparently perfect health for at least four months, and this, though they be kept under conditions less favourable than would occur in nature, the constant handling and fly feedings to which these buck were subject being borne in mind.

5. *Are Antelope Living in the Fly-Area Naturally Infected with Sleeping Sickness?*

Positive evidence on the last query would complete the chain of evidence that antelope living in the fly-areas may act as a reservoir of the virus of Sleeping Sickness. So far it has only been proved that they are "potential" hosts.

The only method by which this query can be investigated is by capturing game in the fly-area and then—

1. Injecting its blood into animals susceptible to *Trypanosoma gambiense* infection.

2. Feeding cages of clean laboratory-bred *Glossina palpalis* on the newly-killed buck and subsequently endeavouring to infect animals susceptible to *Trypanosoma gambiense* with these flies. (The Commission know by observation that *Glossina palpalis* will feed readily on animals newly killed.)

Those who know the local conditions will appreciate the difficulty of carrying out these methods of investigation. It would be necessary for large drives of the buck to be organised in the fly-areas. Several hundred natives would be required. Large numbers of clean laboratory-bred *Glossina* must be available, many normal susceptible animals must be in readiness, and, at the same time, be so situated that they cannot be bitten by the possibly infected wild flies in the neighbourhood. The laboratory work in hand may have to be abandoned for the time being, and some 50 or 60 buck must be captured or killed before reliable information is forthcoming. The sun is hot, the country very difficult, and the exposure to the bites of the fly very great.

It may be said at once that the Commission were only able to shoot two buck on the Lake-shore. The blood of these gave negative results for *Trypanosoma gambiense* when injected into susceptible animals. One of them, however, gave a positive result for *Trypanosoma vivax*. This trypanosome was proved by the Commission* to be not uncommonly carried by wild Lake-shore *Glossina palpalis*. It is therefore not unreasonable to suppose that at least one of the buck shot had been fed on by the Lake-shore fly.

It was recognised at length that occasional week-end shoots by a member of the Commission were quite inadequate. The assistance of the Acting-Governor of Uganda, S. C. Tomkins, Esq., C.M.G., the Acting Principal Medical Officer, Dr. C. Wiggins, of the Uganda Medical Staff, the Provincial Commissioners of Kampala, F. A. Knowles, Esq., C.M.G., and L. H. Cubitt, Esq., and of Sir Apolo Kagwa, K.C.M.G., the Prime Minister, was then sought to aid in organising a series of large drives of wild game in the fly-area in the neighbourhood of the laboratory. We are greatly indebted to these gentlemen for their ready efforts on our behalf, which resulted in a large drive being organised. Unfortunately, the Commission were ordered to leave the country before the drive could take place. The work on these lines, however, is being continued by Dr. R. van Someren, of the Uganda Medical Staff, and Captain A. D. Fraser, Royal Army Medical Corps, who were instructed to take over the laboratory work.

This very difficult question, therefore, still awaits its answer.

Conclusions.

1. Water-buck, bush-buck, and reed-buck can readily be infected with a *human strain* of the trypanosome of Sleeping Sickness by the bites of infected *Glossina palpalis*.

2. One exposure to the bites of infected flies is sufficient to infect an antelope with the virus of Sleeping Sickness.

3. Though the blood of an antelope may be proved to be infected with

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* *Vide* 'Roy. Soc. Proc.,' B, 1910, vol. 82, pp. 63—66.

Trypanosoma gambiense, careful and continued examinations over prolonged periods may fail to reveal the presence of the parasite in the blood.

4. The incubation of the disease (Sleeping Sickness) in antelope is probably seven days.

5. Antelope of the water-buck, bush-buck, and reed-buck species, when infected with the virus of Sleeping Sickness, can transmit the infection to clean laboratory-bred *Glossina palpalis*.

6. This transmission of the infection to clean laboratory-bred flies may occur at least 81 days after the last feed of the infected flies on a buck.

7. *Glossina palpalis*, when infected with the virus of Sleeping Sickness obtained from the blood of infected antelope, are capable of transmitting the virus to susceptible animals.

8. An appreciable percentage of *Glossina palpalis* will become infected with the virus of Sleeping Sickness should these flies feed on antelope suffering from this disease.

9. It follows, from the above conclusions, that antelope living in the fly-areas are "potential" reservoirs of the virus of Sleeping Sickness.

10. No antelope up to the present has been found naturally infected with *Trypanosoma gambiense*.

Experiments to Ascertain if the Domestic Fowl of Uganda may Act as a Reservoir of the Virus of Sleeping Sickness (Trypanosoma gambiense).

By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; and Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, R.A.M.C. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10).

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Introduction.

Birds of various species are very numerous on the shores of Lake Victoria. Of these, cormorants, darters, herons (African grey and purple, and other species), ibises (glossy and sacred), fish-eagles, weaver birds (various species), terns (various species), gulls, geese (Egyptian and spur-winged), plovers (various species), pratincoles, storks, kingfishers and gallinules are the most common. These birds all inhabit areas where the *Glossina palpalis* are numerous, and some evidence is forthcoming that in Nature the fly feeds on avian blood.*

In view of the continued infectivity of the fly in the depopulated areas of the Lake-shore, it is clear that every effort should be made to ascertain the source of such infectivity. Search for a reservoir of the virus of Sleeping Sickness at once suggests itself, and to those acquainted with the fauna of the Lake-shore, an avian host would be included amongst the various species requiring investigation.

Domestic fowls were employed in these experiments. They are readily obtained, easily handled, and the flies feed greedily on them. One species of trypanosome may in Nature only affect one species of avian host; therefore, it follows that whatever the result of this series of experiments may be, that result is only applicable to the fowl. The Commission trust that no wider significance will be attached to the conclusions set forth at the end of this paper: in other words, because a Uganda fowl is or is not a reservoir of *Trypanosoma gambiense*, it does not follow that a cormorant, or other bird, is or is not a reservoir. It would have been better for this reason to have experimented with one or other of the various species of wild Lake-shore birds. The difficulties were, however, too great at the time these experiments were carried out. It is hoped that this paper will stimulate rather than discourage other workers to carry on this line of investigation.

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* 'Roy. Soc. Proc.,' B, 1910, vol. 82, p. 496, Table IV.

In order to discover whether Uganda fowls can act as a reservoir of the virus of Sleeping Sickness, the Commission asked themselves the following questions:—

1. Can fowls be infected with the virus of Sleeping Sickness by the bites of laboratory-bred and laboratory-infected *Glossina palpalis ?*

2. Can fowls transmit the virus of Sleeping Sickness (*Trypanosoma gambiense*) to clean laboratory-bred *Glossina palpalis ?* If this is possible, can the flies so infected convey the virus to normal susceptible animals ?

An answer in the affirmative to those two questions would throw suspicion on a possible avian host. To decide these points, the following experiments were devised and carried out:—

Can Fowls be Infected with the Virus of Sleeping Sickness (Trypanosoma gambiense) by the Bites of Laboratory-bred and Laboratory-infected Glossina palpalis ?

The method adopted to infect the fowls was carried out as follows:—Clean *Glossina palpalis,* hatched out in the laboratory, were given their first feeds on a monkey whose blood contained many *Trypanosoma gambiense.* An interval of at least 24 hours was then allowed to elapse before the infected flies were first fed on the fowl. The fowl was then fed on, for varying periods, by these flies. To make certain the flies were infective, their infectivity was usually tested by their being allowed to bite normal susceptible animals during the experiment. Finally, to discover if the fowl had become infected with *Trypanosoma gambiense* by the bites of the *Glossina palpalis :*—

(*a*) The fowl's blood was examined frequently throughout the experiment for *Trypanosoma gambiense.*

(*b*) The fowl was killed and its centrifuged heart or jugular vein blood was carefully examined microscopically for *Trypanosoma gambiense.*

(*c*) Two or more cubic centimetres of the centrifuged blood were injected into a normal susceptible animal on one or more occasions during the experiment.

Two typical experiments are given in detail.

Experiment 1915 *a*.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1909.				
Nov. 22—25	1—3	Flies fed on *T. gambiense*-infected monkey.		250 laboratory-bred *G. palpalis* used.
„ 26—Dec. 31	4—39	Flies fed on Cock 1926.	—	*T. gambiense*-infected flies found on Dec. 13, 14, 16, 17, 20, and 27.
1910.				
Jan. 1—10	40—49	Flies fed on normal monkey, Experiment 1999, to test their infectivity.		100 flies of this experiment survived to 50th day and were used for Experiment 1915*b*.
„ 11	50	Monkey, Experiment 1999, examined; blood showed *T. gambiense*.	+	Allowing 7 days for the incubation of *T. gambiense* in this monkey, it is seen that the flies infected it on Jan. 4—that is, 4 days after feeding on fowl.

Remarks.—On December 17, 1909, 1 c.c., and on December 22, 3 c.c. of the blood of cock, Experiment 1926, were injected into a normal monkey, Experiment 1954. This monkey remained healthy. On January 10, 1910, the cock was killed, 10 c.c. of its heart's blood were centrifuged, and the "buffy layer" carefully examined for *Trypanosoma gambiense*, with negative results. The whole of the 10 c.c. of blood obtained were then injected into two monkeys: 5 c.c. into monkey, Experiment 1954, and 5 c.c. into monkey, Experiment 2054. The blood of these two animals was examined frequently for a month after these injections. No trypanosomes were ever found and the monkeys remained healthy.

Experiment 2081.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910.				
Jan. 9—14 ...	1—5	Flies fed on *T. gambiense*-infected monkey.		120 laboratory-bred *G. palpalis* used.
„ 15—Feb. 2	6—24	Flies fed on cock, Experiment 2074.	—	Feb. 15, monkey, Experiment 2135, showed *T. gambiense* in its blood.
Feb. 3—7	25—29	Flies fed on normal monkey, Experiment 2135, to see if flies are infective.	+	Feb. 22, monkey, Experiment 2178, showed *T. gambiense* in its blood.
„ 8—14 ...	30—36	Flies again fed on cock, Experiment 2074.	—	Fowl, Experiment 2074, has therefore been fed on by infective *G. palpalis*.
„ 15—19 ...	37—41	Flies fed on normal monkey, Experiment 2178, to see if flies still infective.	+	

Remarks.—Cock, Experiment 2074, was killed on March 16, 1910; 10 c.c. of its heart's blood were taken and centrifuged and carefully examined for *Trypanosoma gambiense*, with negative results. The 10 c.c. were then injected, subcutaneously, into normal monkey, Experiment 2301. This monkey was examined bi-weekly up to April 15, 1910. No *Trypanosoma gambiense* were ever seen, and the monkey remained healthy.

The 13 experiments carried out on these lines are now given in tabular form, in Table I below:—

Table I.

No. of experiment.	No. of flies used.	No. of fowl fed on.	No. of infected flies found during experiment.	Result.	Remarks.
1915 *a*	250	Expt. 1926	7	Negative	Proved infective flies fed on this fowl.
1915 *b*	100	" 2059	1	"	Proved infective flies fed on this fowl.
1915 *c*	50	" 2182	—	"	Doubtful whether infected flies fed on this fowl.
1918	100	" 1927	—	"	Proved infective flies fed on this fowl.
2081	120	" 2074	2	"	Proved infective flies fed on this fowl.
2082	100	" 2087	12	"	Proved infective flies fed on this fowl.
2208	100	" 2209	0	"	Proved infective flies fed on fowl, but no flies dissected.
2325	160	" 2334	2	"	Proved infective flies fed on fowl.
2362	110	" 2363	7	"	Proved infective flies fed on fowl.
2365	300	" 2336	0	"	Flies not dissected, but were proved infective.
2366	150	" 2364	0	"	Flies not dissected, but were proved infective.
2390	180	" 2367	7	"	Proved infective flies fed on fowl.
2395	100	" 2396	1	"	Proved infective flies fed on fowl.

Remarks.—In these experiments it is seen that 1820 laboratory-bred and laboratory-infected *Glossina palpalis* were fed on 13 fowls, with uniformly negative results. With one exception, Experiment 1915 *c*, the flies were proved to be infective by feeding them on animals susceptible to *Trypanosoma gambiense* infection. That the fowls had not become infected with *Trypanosoma gambiense* was proved by carrying out the three tests given above, under headings *a*, *b*, and *c*. In Experiment 2325, the liver, spleen and bone-marrow of Fowl 2334 were also examined for trypanosomes, and 10 c.c. of the pooled pulp injected into a normal monkey. This monkey remained healthy.

A further series of experiments bearing on this question was carried out. These experiments are not so complete. The procedure was as follows:—

A varying number of laboratory-bred *Glossina palpalis* were fed for several days on *Trypanosoma gambiense*-infected antelope. The infected flies were then fed for varying periods on fowls. The fowls were then killed and 2 c.c. of their blood were injected into a normal monkey. The monkey's blood was examined for *Trypanosoma gambiense* bi-weekly for a month after the injection of the fowl's blood. The experiments are given in tabular form.

Table II.

Date.	Days of "cycle" the flies fed on fowl.	Experiment No. of fowl.	No. of flies used.	Did flies subsequently prove to be infective?	Result of inoculation of fowl's blood into normal monkeys.
1910.					
May 9—21	7—19th	Cock 2455	50	No	Negative
„ 13—31	4—22nd	„ 2471	60	Yes (18 p. c.)	„
„ 16—June 1	7—23rd	„ 2479	95	„ (2·1 p. c.)	„
„ 16— „ 1	4—20th	„ 2480	55	„ (15 p. c.)	„
June 2—11.........	10—19th	„ 2518	100	No	„
„ 2—14.........	5—17th	„ 2519	100	„	„
„ 2—17.........	8—23rd	„ 2519	100	Yes (8·8 p. c.)	„
„ 2—11.........	11—20th	„ 2519	100	No	„

Remarks.—The chief criticism against these experiments is the fact that the flies fed on the fowls during the earlier days of the experiments, when the *Glossina palpalis* are usually not infective. However, as results have been recorded by the Commission where *Glossina palpalis* have infected susceptible animals as early as the 18th day of an experiment, it has been decided to publish these experiments.

Can Fowls Transmit the Virus of Sleeping Sickness to clean Laboratory-bred Glossina palpalis? If this is possible, can the Flies so Infected convey the Virus to Normal Susceptible Animals?

In view of the results obtained by the first series of experiments, it would seem somewhat unnecessary to follow this question further.

It has so far been proved that the blood of fowls which have been fed upon by infected *Glossina palpalis* is non-infective when injected into susceptible animals. It has now to be ascertained if the blood of such fowls is also incapable of infecting the fly. This would constitute additional evidence against the fact that fowls act as a reservoir of *Trypanosoma gambiense.*

Cages of laboratory-bred and laboratory-infected *Glossina palpalis,* which were known to be infective, were fed on fowls for a varying number of days. Next, clean laboratory-bred flies were fed on these fowls for several days. The flies were then fed on normal susceptible animals (monkeys), in the endeavour to infect such animals with *Trypanosoma gambiense,* and so prove that the blood of the fowl was infective. The full details of three experiments are given below:—

Experiment 2018.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910.				
Jan. 4—10	1—6	Flies fed on Fowl 1927.		100 laboratory-bred *G. palpalis* used. Fowl, Experiment 1927, has been fed on for 29 days by *T. gambiense*-infected *G. palpalis*.
,, 11	7	Flies starved.		
,, 12—Feb. 23	8—50	Flies fed on Monkey 2061.	—	87 *G. palpalis* dissected throughout this experiment between the 11th and 55th days after flies' first feed on Fowl 1927. All were negative for flagellates. Monkey 2061 examined, with negative result, till March 7.

Experiment 2019.

Date.	y of eri- nt.	Procedure.	Result.	Remarks.
1910.				
Jan. 4—10	–6	Flies fed on Fowl 1926.		100 laboratory-bred *G. palpalis* used. Fowl, Experiment 1926, has been fed on for 29 days by *T. gambiense*-infected *G. palpalis*.
11	7	Flies starved.		
12—Feb. 23	–50	Flies fed on Monkey 2062.	—	80 *G. palpalis* dissected throughout this experiment between the 11th and 55th days after flies' first feed on Fowl 1926. All were negative for flagellates. Monkey 2062 was examined bi-weekly for *T. gambiense* until March 22; no trypanosomes were seen. Monkey remained healthy.

Experiment 2276.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910. Mar. 7—12.........	1—5	Flies fed on Fowl 2087.		200 laboratory-bred flies used. Fowl, Experiment 2087, has been fed on for 18 days by *T. gambiense*-infected *G. palpalis.*
Mar. 13	6	Flies starved.		
„ 14—Apr. 16	7—40	Flies fed on Monkey 2344.	—	116 *G. palpalis* dissected throughout this experiment, between the 23rd and 42nd days after flies' first feed on Fowl 2087. All were negative for flagellates. Monkey 2344 was examined bi-weekly for *T. gambiense* till May 10; no trypanosomes ever seen. Monkey died of broken neck later.

Remarks.—It will be seen that 400 laboratory-bred *Glossina palpalis* fed on the three fowls, Experiments 1927, 1926, and 2087, subsequently failed to infect monkeys with the virus of Sleeping Sickness, and that all the 283 *Glossina* dissected throughout these experiments were negative for flagellates. This absence of flagellates in the flies is interesting, because Fowl 1927 and Fowl 1926 were both found to be naturally infected with a large avian trypanosome. It would thus seem that this fowl trypanosome does not undergo development in *Glossina palpalis*.

Conclusion.

The Uganda fowl cannot act as a reservoir of the virus of Sleeping Sickness.

A Summary of Further Experimental Researches on the Etiology of Endemic Goitre. (Second Series.)

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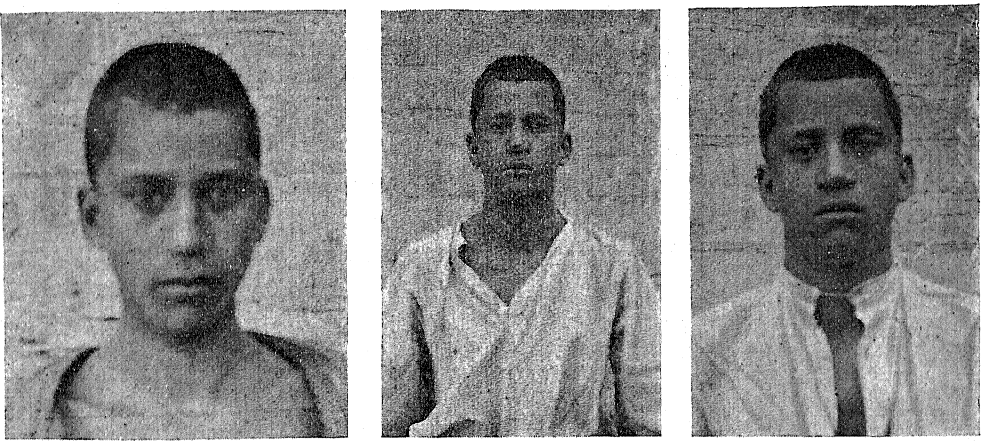
(Communicated by Major Ronald Ross, C.B., F.R.S. Received November 25, 1910,—Read February 2, 1911.)

The object of the research was to test by further experimentation on man the accuracy of the results communicated to the Royal Society on November 26, 1908.* These results afforded evidence that goitre was due to a living organism of disease present in the water of goitrous localities, that the causal factor of the disease was destroyed by boiling, and that it inhabited, in all probability, the intestinal tract of man.

I. Twenty-three individuals, of the average age of 22, consumed the suspended matter of goitre-producing water for periods of from 30 to 55 days. Of these, six showed an increase in size of the thyroid gland which persisted in a more or less well-marked manner up to the end of the experiments. Three others showed a thyroid hypertrophy of a transitory character. (See photos.)

Artificially produced Goitre.



Figures illustrating the artificial production of goitre by means of the suspended matter separated by filtration from goitre-producing water. The figure to the left of the observer represents the subject of the experiment before the consumption of suspended matter was commenced. The remaining figures represent the appearance of the subject thirty days later. Attention is directed to the subject's method of fastening his neck-band, which had fitted comfortably before the experiment was commenced.

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* 'Roy. Soc. Proc.,' B, vol. 81, p. 31.

II. Twenty-three individuals, of the average age of 22, consumed the *boiled* suspended matter of goitre-producing water for periods of from 30 to 55 days. Of these, none showed the slightest tendency to increase in size of the thyroid gland.

III. Seven individuals, of the average age of 22, consumed the filtered goitre-producing water concurrently with the subject consuming the suspended matter of the same water. Of these, none showed the slightest increase in size of the thyroid gland, while three of them, who were sufferers from incipient goitre, showed a marked diminution in size of the organ.

It is pointed out that the enlargement of the thyroid gland which can be experimentally produced in this way is not great, nor is it progressive under the conditions of the experiments. The development of goitre is believed to be largely dependent on certain secondary factors which favour the action of the unknown causal factor of the disease.

These factors are classed as: (1) those directly influencing the thyroid gland, rendering it less able to counteract the action of the toxic agent of goitre without undergoing hypertrophy; (2) those which favour infection; (3) those which favour the action of the virus of the disease at the time of its entry into the body.

IV. The curative action exercised by the lactic ferments when applied to the treatment of cases of incipient goitre is believed to afford additional proof of the view that the organism responsible for the production of goitre has its habitat in the intestinal tract of man. (See photos.)

V. Experiments were carried out on dogs to test the possibility of the communication of the disease to these animals by means of watery extracts from the fæces of goitrous individuals. The results were negative.

It is concluded that—

1. There exists in suspension, in waters which are known to be goitre-producing, an agent which is capable of initiating an hypertrophy of the thyroid gland.

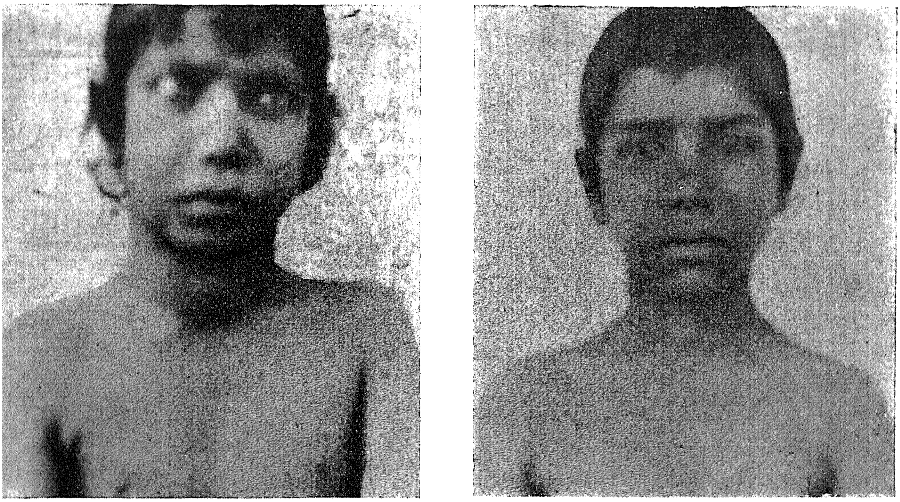
2. This agent is destroyed by boiling and is removed from the water by filtration.

3. This agent is, therefore, either a living organism or a chemical substance the noxious properties of which are destroyed by heat.

4. The incubation period of experimentally produced goitre is usually about 10 to 15 days.

5. Goitre can be cured by the administration of intestinal antiseptics. The lactic ferments exercise a curative action when applied to the treatment of incipient goitres.

TREATMENT OF GOITRE BY LACTIC ACID FERMENTS.



BEFORE. AFTER.

Figures illustrating the action of the *Bacillus Bulgaricus* in the treatment of incipient goitre. The figure to the left of the observer represents the case before treatment; that to the right the same case after 30 days' treatment with "soured milk." Fourteen ounces of "soured milk" were consumed daily. Attention is directed to the loss of subcutaneous fat as a result of the treatment. This result is common to all successful medicinal treatments of goitre, whether by iodine, thymol, or lactic acid ferments.

6. It is very probable that the agent which is responsible for the production of goitre is a living organism parasitic in the human intestine.

7. The disease cannot be communicated to dogs by means of watery extracts from the fæces of goitrous individuals.

The results confirm in detail those which I communicated to the Royal Society on November 26, 1908.

Photographs are given of a case of incipient goitre treated by *B. Bulgaricus* and of the artificial production of goitre by goitre-producing water.

Experiments to Investigate the Infectivity of Glossina palpalis *Fed on Sleeping Sickness Patients under Treatment.*

By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, R.A.M.C. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10); and Dr. R. VAN SOMEREN, Uganda Medical Staff.

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Introduction.

It is well known that there are cases of Sleeping Sickness in man which, under treatment, may enjoy long periods of apparent good health. In these cases the individual is often able to live an active life, and insists on being at liberty to go about as he pleases. In Uganda care is taken to prevent such cases entering the fly-areas; but the difficulty in maintaining a supervision strict enough to check occasional visits to these areas is obviously great, and in other countries may be insuperable, so that the question as to how drug treatment can influence the infectivity of these patients to the fly may be considered to be of importance.

Other points arise from the consideration of this question, viz.:—Can one or more doses of a trypanocidal drug render a Sleeping Sickness patient non-infective to *Glossina palpalis?* Does prolonged treatment during any stage of the disease render cases of Sleeping Sickness innocuous to the fly? If it be proved that the treatment of Sleeping Sickness patients by certain drugs does not prevent them infecting *Glossina palpalis*, then what percentage of cases give a positive result? and what percentage of flies are infected from these cases, as compared with the number of flies infected from untreated cases?

To answer these questions it is necessary to classify under various headings the patients who are the subjects of this paper; to state whether treated or untreated; if the latter, to give details concerning the nature of the treatment, the duration of treatment, and the total quantity of drugs received. The presence or absence of *Trypanosoma gambiense* in the blood or lymph glands of each patient should also be noted.

The classification of patients, according to the stage of the disease in which they present themselves, is that adopted by Dr. A. D. P. Hodges, C.M.G., Principal Medical Officer, Uganda, in the "Progress Report on the Uganda Sleeping Sickness Camps," from December, 1906, to November 30, 1908.

Class A.—Those who are apparently in good health: gland enlargement with trypanosomes present in the glands.

Class B.—Those in whom there are early signs from which the existence of Sleeping Sickness may be reasonably suspected.

*Class C.**—Those in whom clinical signs are well marked.

*Class D.**—Advanced cases.

These experiments were carried out from January to March, 1910. Of the 33 patients under observation,

6	belonged to	Class	A.
19	,,	,,	B.
7	,,	,,	C.
1	,,	,,	D.

The methods adopted to test the degree of infectivity of patients to the fly in different stages of the disease were: (1) By mechanical transmission from Sleeping Sickness patient to normal monkey, and (2) by transmission of the disease from Sleeping Sickness patient to normal monkey after the parasite had completed its development in the fly.

1. *By Mechanical Transmission from Sleeping Sickness Patient to Normal Monkey.*

The procedure in testing the first method was as follows:—A cage of laboratory-bred flies was first placed for two minutes upon the bare shoulders of a patient suffering from Sleeping Sickness, which was time enough to allow the majority of the flies to draw some blood, but not enough to satisfy them. After an interval of five minutes the cage was transferred to the abdomen of a normal monkey, where it remained for two minutes, by which time nearly all the flies had completed their feed. This procedure was repeated daily for six days.

Ten of the patients under experiment had been treated with soamin and perchloride of mercury administered by subcutaneous injection. Two patients, who had been under treatment for one year, had received several doses of atoxyl, in addition to soamin and mercury.

Trypanosomes had been found at one time in the glands of all the patients, but no trypanosomes were found in their blood during the time the flies were being fed upon them.

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* Classes C and D are not now differentiated.

Table I.—Transmission of *Trypanosoma gambiense* from Sleeping Sickness Patients to Healthy Monkeys by Interrupted Feeding.

No. of experiment.	Class of patient.	If treated.	Duration of treatment.	No. of doses.	No. of flies fed.	No. of times flies fed.	Result.
2014	A	Yes	7 days	2	75	6	—
2137	B	"	7 "	2	65	7	—
2144	C	"	7 "	2	75	6	—
2146	B	"	7 "	2	65	6	—
2013	A	"	30 "	4	75	6	—
2027	B	"	30 "	4	75	6	—
2029	B	"	30 "	4	75	6	—
2150	C	"	30 "	4	70	7	—
2152	C	"	30 "	4	50	7	—
2158	B	"	30 "	4	60	6	—
2139	B	"	1 year	?	70	6	—
2154	B	"	1 "	?	50	6	—
2012	B	No	Nil	Nil	75	6	—
2281	B	"	"	"	100	6	—
2284	B	"	"	"	50	6	—

Remarks.—These experiments are all negative—a not unexpected result now that it is known that the mechanical transmission of Sleeping Sickness may be a somewhat rare event in Nature. The question as to whether the medicinal treatment of Sleeping Sickness patients has any preventive influence on this method of transmission remains unanswered. It may, however, be noted that in the two untreated cases, viz., Experiment 2281, Class B, and Experiment 2284, Class B, the disease was not transmitted mechanically, but, as will be seen later (Table III, Experiments 2280 and 2298), was conveyed after a period of development of the parasite within the fly.

2. *By Transmission of the Disease from Sleeping Sickness Patient to Normal Monkey after the Parasite had Completed its Development in the Fly.*

The procedure adopted in the second method for transmitting the disease from a Sleeping Sickness patient to a monkey was as follows:—A cage of laboratory-bred flies was fed daily for five or six days upon a patient suffering from Sleeping Sickness. They were then starved for 48 hours, and subsequently fed daily for 50 days upon a normal monkey.

In most of these experiments the flies were dissected and examined for flagellates after the period of feeding upon the normal monkey. The monkey's blood was subsequently examined twice weekly for one month after the last date on which the flies were fed upon it.

Trypanosomes had been found at one time in the glands of all these patients, but no trypanosomes were found in their blood during the time the flies were being fed upon them.

In the following tables all the details bearing on each class of patient are given:—

Table II.—Transmission of *Trypanosoma gambiense* from Sleeping Sickness Patients, Class A, to Healthy Monkeys, an Interval of Time being allowed for the Development of the Trypanosomes in the Fly.

No. of experiment.	Duration of treatment.	No. of doses.	No. of flies fed on patient.	No. of days flies fed on patient.	No. of days flies fed on monkey.	Result.	Remarks.
1941	7 days	2	125	6	50	—	Monkey remained healthy.
1973	7 "	2	100	6	50	—	"
1969	1 month	4	100	7	50	—	"
1995	1 "	4	130	4	50	—	"
2240	2½ years	?	75	4	50	—	"
2252	2½ "	?	80	4	50	—	"

Remarks.—Reference to Table II will show that six patients in Class A stage of the disease were under treatment. Two of these had been under treatment for one week, and had each received two injections of soamin, 0·01 gramme, combined with perchloride of mercury, 0·02 gramme. Two had been under treatment for one month, and had received four injections of soamin, 0·01 gramme, and perchloride of mercury, 0·02 gramme, per dose. Two cases had been under treatment for two and a-half years: the total quantity of drugs received being, in one case, atoxyl, 16·2 grammes, perchloride of mercury, 2·4 grammes, and orpiment, 8·6 grammes; the other case had received 18·8 grammes of atoxyl, 2·4 grammes of perchloride of mercury, and 8·6 grammes of orpiment.

Sleeping Sickness was not transmitted from any of these patients to monkeys by the flies.

Two hundred and seventy-three flies were dissected between the 4th and 51st days after feeding upon infected patients, but no flies were found infected with flagellates.

Only one patient belonging to Class D was available for these experiments. This patient had been under treatment for one and a-half years, and had been treated with a total quantity of 13·4 grammes atoxyl, 0·4 gramme perchloride of mercury, and 6·5 grammes orpiment.

Flies were not infected from this case, and the disease was therefore not transmitted to a normal monkey.

Table III.—Transmission of *Trypanosoma gambiense* from Sleeping Sickness Patients, Class B, to Healthy Monkeys, an Interval of Time being allowed for the Development of the Trypanosomes in the Fly.

No. of experiment.	Duration of treatment.	No. of doses.	No. of flies fed on patient.	No. of days flies fed on patient.	No. of days flies fed on monkey.	Result.	Remarks.
1993	7 days	2	100	4	50	–	Monkey remained healthy.
2091	7 „	2	50	5	50	–	”
2094	7 „	2	60	5	50	–	”
2170	7 „	2	60	6	50	–	”
1975	30 „	4	100	5	50	–	”
1987	30 „	4	65	5	50	–	”
1991	30 „	4	90	4	50	–	”
2068	30 „	4	65	6	50	–	”
2070	30 „	4	80	6	50	–	”
2107	30 „	4	65	6	50	–	”
2195	30 „	4	50	6	50	–	”
2248	30 „	4	65	4	50	–	”
2096	1 year	8	60	5	50	–	”
2109	1 „	9	65	5	50	–	”
2172	1 „	8	60	5	50	–	”
2193	1 „	9	60	6	50	–	”
2254	2¼ years	?	80	4	50	–	”
2256	2¼ „	?	60	4	50	–	”
2258	3½ „	?	60	4	50	–	”
1943	Nil	Nil	65	6	50	–	”
1957	”	”	100	4	50	–	”
1967	”	”	110	6	50	–	”
2048	”	”	50	6	50	–	”
2280	”	”	130	4	50	+	*T. gambiense* appeared in blood.
2298	”	”	80	3	50	+	”

Remarks.—In Table III, Class B, 25 experiments from 19 different patients are recorded. Six of these were from untreated cases.

Sleeping Sickness was transferred from two untreated patients to normal monkeys. The flies belonging to these experiments were not dissected.

The remaining four experiments from untreated cases were negative, both as regards infection of the flies (190 dissected) and the conveyance of the disease to monkeys.

Of the 19 negative experiments from cases under treatment, four were from two patients who had been under treatment for one week. They had each received two injections of soamin in 1-gramme doses, combined with 0·02 gramme of perchloride of mercury. Eight experiments were from patients who had been one month under treatment. They had each received four injections (soamin 1 gramme, and perchloride of mercury 0·02 gramme, per dose). Four experiments were from two cases who had been under treatment for 11 months. One of these had received injections amounting in all to soamin 8 grammes, $HgCl_2$ 0·10 gramme, and orpiment 2 grammes. The other patient had received a total amount of soamin 9 grammes, $HgCl_2$ 0·10 gramme, and orpiment (?). Two experiments were from patients who had been under treatment for two and a-quarter years. They had each received a total quantity of drugs amounting to atoxyl 19·7 grammes, $HgCl_2$ 0·06 gramme, orpiment 4·60 grammes, and soamin 4 grammes. One experiment was from a patient who had been three and a-half years under treatment, and had received during the last 18 months atoxyl 9·5 grammes, soamin 7·5 grammes, and orpiment 4·6 grammes.

From these 19 negative experiments 509 flies were dissected between the 4th and 52nd days after their last infected feed. No flagellates were found in any of them.

Table IV.—Transmission of *Trypanosoma gambiense* from Sleeping Sickness Patients, Class C, to Healthy Monkeys, an Interval of Time being allowed for the Development of the Trypanosomes in the Fly.

No. of experiment.	Duration of treatment.	No. of doses.	No. of flies fed on patient.	No. of days flies fed on patient.	No. of days flies fed on monkey.	Result.	Remarks.
2103	7 days	2	60	6	50	—	Monkey remained healthy.
2168	7 "	2	63	6	50	—	"
2250	7 "	1	100	4	50	—	"
2034	30 "	4	100	3	50	+	*T. gambiense* appeared in blood.
2098	30 "	4	50	5	50	—	Monkey remained healthy.
2105	30 "	4	65	4	50	—	"
2189	30 "	4	60	7	50	—	"
2191	30 "	4	65	7	50	—	"
2244	2½ years	?	120	4	50	—	"
2242	Nil	Nil	85	4	50	—	"

Remarks.—In Table IV, Class C, 10 experiments are recorded. One of these was from an untreated case with large numbers of trypanosomes in the cervical glands, but none detectable in the blood.

The monkey was not infected by flies fed upon this patient, and so the flies were not dissected.

Of the 9 experiments from treated cases, Class C, three were from two patients who had been under treatment for one week, of whom one had been given a single dose of soamin 2 grammes, and $HgCl_2$ 0·04 gramme. The other patient had received two doses.

The experiments performed from these patients were negative as regards infection of both monkeys and flies.

Three patients had been under treatment for one month. Each of these patients had been given injections of soamin and perchloride of mercury, amounting to 2 grammes of the former drug and 0·08 gramme of the latter. One of these experiments was positive, both as regards infecting the flies and transmitting the disease to monkeys.

Seventy-five flies were dissected from the positive experiment, 2034. Of these, four flies were found to be infected with *Trypanosoma gambiense* on the 28th, 39th, 40th, and 46th day respectively after their last infected feed. The four flies found to be infected with trypanosomes were injected into a susceptible animal, which subsequently developed Sleeping Sickness.

It is obvious that the results obtained from these experiments, though giving us some information, are insufficient to warrant a decisive answer to the questions before us.

With regard to the first query: Can treatment of Sleeping Sickness patients by the drugs named influence the infectivity of the fly? Omitting the mechanical transmission experiments, we may say that out of the 35 experiments only one produced a positive result, *i.e.* flies were infected and the disease transmitted from a Sleeping Sickness patient under treatment to a normal monkey. The case referred to above will also entitle us to express an opinion as to whether one or more doses of a certain trypanocidal drug can render a patient non-infective to the fly. It will be seen that this case had been under treatment for one month, and had been injected with four doses of trypanocidal drugs. The tsetse flies were infected 25 days after the last of the four injections had been administered.

Therefore, it may be said that to treat Sleeping Sickness patients with injections of soamin and perchloride of mercury, as specified, does not necessarily cause their blood to be incapable of infecting *Glossina palpalis* with *Trypanosoma gambiense*, and that flies so infected can transmit the disease to monkeys.

The percentage of flies infected from untreated cases was not ascertained, as only 190 flies were dissected, at various intervals, after feeding on their patients, and none were found infected.

A total of 857 flies were dissected, at different periods, after having fed upon treated cases, and four of these were found to be infected, *i.e.* a percentage of 0·46. These four flies all came from a cage containing 100 *Glossina palpalis* which had been fed on three occasions upon the only treated case that proved infective to the flies, *vide* Table IV, Experiment 2034. This patient therefore infected 4 per cent. of flies—an interesting and important fact.

It was hoped that patients under the influence of trypanocidal drugs would be incapable of infecting tsetse flies. Unfortunately, this experiment (2034) is sufficient to show that Sleeping Sickness patients under treatment with arsenical compounds, etc., are not always innocuous to the fly, and should not be allowed their freedom to wander about the country during intervals of comparative good health, but must be kept completely and permanently isolated from all possible contact with the fly.

Conclusions.

1. *Glossina palpalis* fed on natives suffering from Sleeping Sickness, and untreated by drugs, may become infected and be capable of transferring the disease to healthy animals.
2. *Glossina palpalis* fed on natives suffering from Sleeping Sickness, and treated by arsenic and other drugs, may also become infected and be capable of transferring the disease to healthy animals.

Experiments to Ascertain if Trypanosoma gambiense *during its Development within* Glossina palpalis *is Infective.*

By Colonel Sir David Bruce, C.B., F.R.S., A.M.S.; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, R.A.M.C.; and Captain F. P. Mackie, I.M.S. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10).

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It will be remembered that the injection of chopped-up tsetse flies (*Glossina morsitans*) a short time after feeding on an infected animal, did not give rise to nagana. As this was thought to be an interesting fact, and difficult of explanation, experiments were carried out on the same lines with *Trypanosoma gambiense* and *Glossina palpalis.*

If tsetse flies (*Glossina palpalis*) are fed upon an animal whose blood contains *Trypanosoma gambiense,* the trypanosomes can be found living within the intestines of some of the flies for several days after they were ingested. In a small percentage (0·5 to 2·0 per cent.) of flies so infected, active trypanosomes may be found swarming in their intestines on any day between the seventh and the fiftieth day, or even longer, after they have been fed upon an infected animal.

A series of experiments were undertaken, to ascertain if *Trypanosoma gambiense* retained its power of causing Sleeping Sickness when inoculated, subcutaneously, into monkeys, throughout its period of multiplication within the fly, especially during the interval of some 20 days in which the bites of infected flies are harmless. In some of these experiments Lake-shore flies were used; in others, laboratory-bred flies.

The flies were fed upon a monkey whose blood contained many trypanosomes. After a pre-determined time had elapsed, the wings and legs of the infected flies were cut off, and the bodies were either chopped up and brayed in a mortar with saline solution (0·8 per cent.), or the alimentary canal alone was removed. In many instances the gut was proved by microscopical examination to be heavily infected with trypanosomes before it was inoculated into a monkey. In flies thus found to be infected, the salivary glands also were carefully removed and washed thoroughly in several changes of normal saline solution. They were then broken up and injected, by means of a sterile syringe, into the subcutaneous tissue of the groin of a monkey.

The following table shows the result of the individual experiments as carried out with Lake-shore flies:—

Table I.—*Glossina palpalis* and *Trypanosoma gambiense.*

Date.	Expt. No.	No. of hours after infected feed.	No. of flies injected.	Result.	Incubation period in monkey.
1909.					
Mar. 10	521	½ hour	2	+	8 days.
Jan. 6	420	¾ "	1	—	
" 6	421	1 "	2	—	
Feb. 18	518	1 "	2	—	
Mar. 19	604	1 "	2	+	6 "
" 19	605	1½ hours	2	+	6 "
Feb. 18	519	2 "	2	—	
June 22	1038	3 "	1	+	6 "
" 22	1043	3 "	1	+	6 "
Mar. 29	622	4 "	8	+	7 "
June 11	984	4 "	5	+	8 "
" 16	1009	4 "	5	+	6 "
" 22	1039	6 "	1	+	6 "
" 22	1044	6 "	1	+	6 "
" 22	1045	7 "	1	+	9 "
" 22	1041	8 "	1	+	9 "
" 22	1046	8 "	1	+	10 "
" 22	1042	9 "	1	+	9 "
" 22	1047	9 "	1	+	9 "
" 26	1154	16 "	1	—	
" 26	1155	16 "	1	—	
" 26	1156	17 "	1	+	13 "
" 26	1157	17 "	1	—	
" 26	1158	18 "	1	+	9 "
" 26	1159	18 "	1	+	13 "
Mar. 20	600	19 "	5	—	
June 26	1161	19 "	1	—	
Mar. 4	524	23 "	6	—	
" 5	585	44 "	6	—	

Remarks.—Twenty-nine experiments were carried out with Lake-shore flies and *Trypanosoma gambiense.* Eighteen of these experiments yielded a positive result, and were almost uniformly successful up to 18 hours after the infected feed. It seemed to be immaterial whether the fly was introduced whole or whether the alimentary canal alone was injected. In several instances where the gut of the fly was examined in the fresh state, swarms of flagellates natural to the fly, *i.e. Trypanosoma grayi,* were found, but their presence did not appear to modify the virulence of *Trypanosoma gambiense.*

The following table shows the result of the experiments as carried out with laboratory-bred flies:—

Table II.—*Glossina palpalis* and *Trypanosoma gambiense.*

Date.	Expt. No.	No. of hours or days after infected feed.	No. of flies injected.	Result.	Incubation period in monkey.
1909.					
Aug. 24	1545	1 hour	1	+	7 days
" 24	1546	2 hours	1	+	7 "
Feb. 18	520	4 "	2	—	
Aug. 24	1547	4 "	1	+	7 "
Sept. 14	1681	1 day	3	—	
" 23	1756	2 days	3	+	12 "
" 26	1723	3 "	15	—	
Oct. 2	1713	4 "	10	—	
" 3	1783	5 "	11	—	
" 4	1752	6 "	10	—	
*Sept. 28	1721	7 "	1	—	
*Nov. 16	1906	8 "	2	—	
Oct. 7	1788	9 "	10	—	
*Nov. 17	1909	9 "	1	—	
Oct. 8	1795	10 "	8	—	
" 9	1799	10 "	8	—	
*Nov. 18	1911	10 "	1	—	
* " 19	1913	11 "	2	—	
* " 29	1923	11 "	1	—	
Oct. 11	1807	13 "	5	—	
Nov. 26	1864	14 "	30	—	
*Nov. 15	1905	14 "	2	—	
*Oct. 3	1781	15 "	1	—	
" 27	1865	15 "	12	—	
" 19	1838	16 "	34	—	
" 29	1872	17 "	12	—	
*Dec. 13	1939	17 "	1	—	
Oct. 30	1873	18 "	12	—	
*Dec. 14	1940	18 "	2	—	
Nov. 1	1877	20 "	12	—	
* " 16	1951	20 "	1	—	
Nov. 2	1879	21 "	20	—	
" 4	1886	23 "	15	—	
Dec. 5	1887	24 "	10	—	
* " 20	1958	24 "	1	+	8 "
" 6	1889	25 "	22	—	
*Sept. 28	1749	28 "	1	—	
* " 29	1759	28 "	1	+	11 "
*Nov. 9	1866	30 "	1	+	12 "
" 4	1884	34 "	1	—	
* " 11	1897	36 "	2	+	5 "
* " 16	1907	37 "	1	—	
* " 4	1885	42 "	1	—	
*Oct. 8	1791	46 "	2	+	9 "
*Nov. 3	1881	49 "	1	—	
* " 11	1898	51 "	1	—	
* " 4	1856	53 "	1	—	

Remarks.—In these experiments the intestines only of the infected flies were inoculated. In the experiments marked with an asterisk the gut was first proved, by microscopical examination, to contain swarms of living *Trypanosoma gambiense,* and then immediately injected into a monkey. It will be seen there are 47, experiments in which a total of 296 laboratory-bred flies were inoculated, subcutaneously, into monkeys at various intervals after the flies had fed upon blood infected with *Trypanosoma gambiense.* The injection into a monkey of a single fly, one, two, and four hours after an infected feed, caused Sleeping Sickness. Three flies inoculated two days after their infected feed produced a like result. Between the 2nd and the 24th days after the flies had fed on infected blood, 249 of them were inoculated; the result was negative in every case, although 15 of these flies proved, by microscopical examination, to be swarming with living *Trypanosoma gambiense* at the time of inoculation. In 13 experiments, 14 flies proved, microscopically, to be infected, were inoculated, between the 24th and 53rd days after they had fed upon an infected animal, and in five of these a positive result was obtained.

The following table shows the result of subcutaneous injections of washed salivary glands dissected from laboratory-bred flies whose intestines contained swarms of *Trypanosoma gambiense* :—

Table III.—*Glossina palpalis* (Salivary Glands) and *Trypanosoma gambiense.*

Date.	Expt. No.	No. of days after infected feed.	No. of flies from which glands injected.	Result.	Incubation period in monkey.
1910. Apr. 14	2376	2 days	3	—	
„ 15	2376	3 „	1	—	
„ 17	2376	6 „	2	—	
„ 18	2376	7 „	3	—	
„ 22	2394	8 „	2	—	
„ 25	2394	11 „	2	—	
Jan. 26	2099	14 „	2	—	
„ 31	2114	21 „	1	—	
Feb. 2	2114	23 „	1	—	
„ 7	2148	28 „	1	—	
„ 9	2148	30 „	1	—	
„ 13	2177	36 „	4	+	8 days.

Remarks.—The results of the microscopical examination of the salivary glands show that trypanosomes do not appear in the glands until about the 30th day. Out of 12 experiments 11 were negative, and one was positive. The glands from 19 infected flies were injected subcutaneously, between the 2nd and 30th days after the flies had fed upon an infected animal; the results were all negative. In the positive experiment the salivary glands from 4 infected flies were injected into a healthy monkey 36 days after the flies had fed on a monkey suffering from Sleeping Sickness, with the result that the former animal contracted the disease.

Conclusions.

1. *Trypanosoma gambiense* may retain their virulence, as ascertained by direct inoculation into susceptible animals, for a period of two days after they are ingested by *Glossina palpalis.*

2. After the trypanosomes have been within the gut of the fly for two days the power of infecting animals with Sleeping Sickness, when inoculated subcutaneously, is lost for a period of 22 days.

3. *Trypanosoma gambiense* regains the power of infecting by direct inoculation after it has been about 24 days within the intestine of the fly.

4. The number of days, during which the virulence of the trypanosomes contained in the fly is lost, roughly coincides with the time that the infected fly is incapable of transmitting Sleeping Sickness by biting susceptible animals.

5. There is some evidence that the salivary glands of the fly are invaded by virulent forms of the parasite 36 days after the fly has fed upon infected blood.

Experiments to Ascertain if Certain Tabanidæ Act as the Carriers of Trypanosoma pecorum.

By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S., and Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, R.A.M.C. (Sleeping Sickness Commission of the Royal Society, 1908–10).

(Received December 20, 1910,—Read March 2, 1911.)

[PLATE 16.]

INTRODUCTION.

The study of the trypanosome diseases of domestic animals in Uganda was a branch, and an important one, of the work undertaken by the Commission. Among other points arising in this investigation, the finding, if possible, of a carrier of the various trypanosome diseases was necessary. In the 'Proceedings'* of the Royal Society, the Commission published a paper entitled "Trypanosome Diseases of Domestic Animals in Uganda. I.—*Trypanosoma Pecorum.*" This paper gives full details as to the definition, morphological and cultural characteristics, and distribution in Africa, of *Trypanosoma pecorum.* A few experiments and experiences of the Commission were also given, which led to the belief that possibly Tabanidæ were the common carriers in Africa of the disease caused in cattle by this trypanosome.

With the object of gaining further knowledge on this important problem, the Commission worked in Uganda from January to July, 1910, on the following lines:—

1. An investigation of the biting flies occurring in the vicinity of the laboratory at Mpumu, Chagwe, Uganda.
2. A study of the natural history of these biting flies.
3. Transmission experiments with these flies.
4. A study of the flagellates, if any, natural to these flies.

1. AN INVESTIGATION OF THE BITING FLIES OCCURRING IN THE VICINITY OF THE LABORATORY AT MPUMU, CHAGWE, UGANDA.

No systematic search was made for blood-sucking flies in this neighbourhood till January, 1910. Up to this date the Commission had identified some four different species in the vicinity of Mpumu.

An outbreak of trypanosome disease in cattle having occurred in

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* B, 1910, vol. 82, p. 468.

September, 1909,* in the valleys surrounding Mpumu hill, it was decided to make a prolonged and careful search for biting flies in these valleys. Boys were employed whose sole duty it was to hunt daily for these flies, cattle accompanying the boys to act as a bait for the flies. This more systematic search produced unexpected results.

In the place of only three or four varieties of biting flies, no less than 12 species of Tabanidæ, five species of Hæmatopota, two different Chrysops, a new and very handsome Rhinomyza, and *Glossina palpalis*, were captured within a 3-mile radius of the laboratory. These flies were kindly identified for us by Mr. E. E. Austen, F.L.S., to whom specimens were sent, and for whose help and courtesy the Commission is much indebted. The full list is here given, as it will probably be of value to future workers in the Mpumu laboratory, and of interest to entomologists.

Tabanidæ.

Tabanus secedens (Walk.), very numerous.
Tabanus fuscomarginatus (Ricardo), very numerous.
Tabanus thoracinus (Pal. de Beauv.), very numerous.
Tabanus par (Walk.), fairly numerous.
Tabanus tæniola (Pal. de Beauv.), fairly numerous.
Tabanus socialis (Walk.), fairly numerous.
Tabanus variatus (Walk.), fairly numerous.
Tabanus fasciatus (Fabr.), sub. sp. *niloticus* (Austen).
Tabanus insignis (Lw.), scarce.
Tabanus variabilis (Lw.), scarce.
Tabanus obscurissimus (?), scarce.
Tabanus irroratus (Surcouf), rare.

Hæmatopota.

Hæmatopota ugandæ (Ricardo). *H. vittata* (Lw.), syn. *pulchrithorax* (Austen). *H. fusca* (Austen). *H. brunnescens* (Ricardo). *H. similis* (Ricardo).

(All numerous.)

Chrysops.

Chrysops funebris (Austen). *C. silacea* (Austen).

Glossina.

Glossina palpalis (Rob.-Desv.).

Rhinomyza.

Rhinomyza perpulcra (Austen).

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* 'Roy. Soc. Proc.,' B, 1910, vol. 82, pp. 476—478.

This part of the investigation made two facts clear to the Commission. Firstly, that experiments to ascertain a carrier of any of the trypanosome diseases of domestic animals in Africa must necessarily be both prolonged and difficult. Secondly, that the distribution of the different species of biting flies in any country cannot be mapped out by a travelling entomologist. Accurate maps can only be made by the whole-hearted co-operation of those officials and settlers who are resident in the country for long periods. The observations should, where possible, be made daily, and continued for at least one year in the same locality.

2. A STUDY OF THE NATURAL HISTORY OF THE BITING FLIES FOUND IN THE NEIGHBOURHOOD OF MPUMU.

It should be clearly understood that whatever observations are given below refer to the natural history of these flies as observed in this locality only. Elsewhere difference in climatic and other conditions may give rise to variations in the habits and life-history of these flies.

Glossina palpalis.

The habits of the Glossina in this neighbourhood do not vary from the same species on the Lake-shore. Their presence was, however, both interesting and disquieting, as the Kasala stream, where they were captured, is at least 6 miles from the nearest point of Lake Victoria. Further, the stream has no connection whatever with this Lake. Rising in a narrow belt of forest at the foot of Mpumu hill, it flows through forest along the north-west face of the hill and then joins the Sezibwa River, which trends northwards, running parallel with the Nile, to empty into Lake Chioga. The Glossina were very scarce, three or four only being caught monthly between January and July, 1910. They were all caught at one spot—a ford of the stream, situated a mile or so from the laboratory. No tsetse-flies were seen in any other part of the stream. It is possible these flies had found their way down from the hill, since it was by no means an uncommon circumstance for some flies to escape from their cages when brought up by the fly-boys. Escaped flies were found on several occasions on different parts of the hill.

The Tabanidæ.

Habitat.—Narrow belts of dense tropical forest, which commonly mark the windings of a flowing stream. The two essential factors appear to be deep shade and water, not necessarily running water. These flies are not found on grass-covered hill-tops.

Habits.—Emerging from the upper branches of the forest trees, on which they presumably shelter during the night, these flies commence feeding about 7 A.M. if the morning be fine with plenty of sunshine. They cease to feed about 6 P.M. Their most active hours are from 11 A.M. to 1 P.M., viz., the hottest part of the day. From 6 A.M. to 8 A.M., and again from 4 P.M. to 6 P.M., they are sluggish and do not feed readily. In wet, cold, or dull weather no flies are to be seen as a rule. They bite cattle and man freely, but much prefer the former, and will rarely attack man in the presence of cattle. Their bite is like the stab of a sharp lancet, and draws a free trickle of blood from both oxen and man. They have followed cattle a distance of 3 miles to the laboratory, but invariably left the hill-top soon after their arrival, or died in sheltered spots about the houses a few days after their arrival.

Variation in Incidence.—This phenomenon is the most striking characteristic of the Tabanidæ. A forested stream known to be free from a certain species of Tabanus one day will be infested with that species during the next two or three days. This eruption, if it may be so called, appears to be simultaneous over a given area, and may be explained by the flies all arriving at maturity about the same day. This tends to show that the females all lay their batches of eggs about the same time. Further, it is not unreasonable to date the commencement of the fly's winged life from this time, and so, watching carefully for their final disappearance, we arrive, probably with some degree of accuracy, at the length of life of the fully-developed fly. The disappearance of the fly is almost as sudden as their eruption; in some three days a locality previously swarming with a certain species of Tabanus will be quite free from this species. In this manner the Commission have observed carefully three species of Tabanidæ, viz., *Tabanus secedens*, *Tabanus fuscomarginatus*, and *Tabanus thoracinus*. In these cases, on the above computation, the limit of life of these flies is as follows:—*Tabanus secedens*, four months; *Tabanus fuscomarginatus*, two months; *Tabanus thoracinus*, four months. At least two such eruptions of the same fly may occur in the same locality in the same year.

The Tabanidæ.

Sex.—During the seven months (January–July, 1910) these experiments were taking place, many thousand Tabanidæ were captured, but no male of any of the 12 different species was ever seen or taken.

The Hæmatopota.

Habitat.—The Hæmatopota have a more extensive range than the Tabanidæ. They are to be found in the same localities as the Tabanidæ, and also, in large numbers, in open swampy areas where few or no trees grow. The rank grass, which grows waist-high in these swamps, appears to satisfy what need they may have for shade. Another very favourite locality for these flies is a road or ford crossing streams and swamps. The fly is absent from dry, open, grass-covered hill tops.

Habits.—The hours of greatest activity, and the feeding times of this genus, correspond to those given above for the Tabanidæ. They have a sluggish flight, and attack man more readily than do the Tabanidæ. When they settle on their victim they rarely bite at once, but usually walk about slowly for a few seconds before inserting their proboscis. They will follow cattle or man for a mile or two.

Sex.—No male fly was ever captured by the Commission during the time these observations were taking place.

The Chrysops and the Rhinomyza.

These flies were comparatively scanty in the neighbourhood, and no accurate observations were made of their natural history. All were caught at the ford on the Kasala stream, at the foot of Mpumu hill. *Rhinomyza perpulcra* appeared to be very localised in its distribution, as it was never seen elsewhere in the vicinity. It was usually captured whilst sunning itself on stones in the stream, or whilst it was biting natives washing clothes at this ford. Its habitat appeared to be the densely forested banks of the ford. Mr. Austen informs us that it is a new African species.

3. EXPERIMENTS TO ASCERTAIN IF TABANUS SECEDENS (WALK.), TABANUS FUSCOMARGINATUS (RICARDO), OR TABANUS THORACINUS (PAL. DE BEAUV.), ARE CAPABLE OF TRANSMITTING TRYPANOSOMA PECORUM FROM SICK TO HEALTHY OXEN.

The experiments were carried out between March and August, 1910. The above-named species were used, as they were the most common among the Tabanidæ during this period. Only wild flies were employed, as the Commission never succeeded in finding the eggs or larvæ of these flies. The species of Tabanus used in any experiment, and the duration of the experiment, were thus entirely dependent on the seasonal incidence of the fly in the neighbourhood.

Endeavours were at first made to carry out the experiments in the laboratory on Mpumu hill. Cages, some 12 inches square, were made, with sides of wood, the top and bottom consisting of wide-meshed mosquito netting. The captured flies were placed in these cases—about 50 flies per cage—the cages being kept over water in the laboratory when the flies were not being fed. The daily temperature of the laboratory during the days this method was tried was very constant, being 70°·2 F. (21°·2 C.) in the morning at 8, and 80°·2 F. (26°·8 C.) in the afternoon at 4. The flies were placed on oxen and monkeys for 20 minutes once daily, to give them ample opportunity to feed.

This method was not successful, the flies—*Tabanus secedens* (Walk.)—all dying within four days of being caged, the majority within 48 hours. When placed on an ox, within 12 hours of capture, a few flies fed greedily. After 24 hours in the cages no fly was observed to bite. Monkeys were never bitten by the flies, though they would feed on man as readily as on the ox when freshly caught. If shaded from direct sunlight, the flies refused to feed.

It was decided to build a fly-proof kraal in the natural haunts of the fly, in the hope that the Tabanidæ would live longer and feed more readily. The Kasala stream at the foot of Mpumu hill was chosen as the site of the kraal. The kraal was divided into two compartments, which were made fly-proof from each other and from the outside. In one compartment no flies of any species had access. Into the other compartment the species of Tabanus under experiment were liberated—50 flies per day, on an average. In the kraal four calves were kept; three normal and one calf infected with *Trypanosoma pecorum*. The infected calf and two of the normal calves were taken from the fly-free compartment into the compartment containing the Tabanidæ for three hours daily. The hours chosen were 11 A.M. to 1 P.M., and 3 P.M. to 4 P.M., when the sun was hot and shining on the kraal. Every opportunity was thus given to the flies to feed on both the infected and the healthy calves. The other normal calf was utilised as a control, being kept continuously in the fly-free compartment and so protected absolutely from the bites of blood-sucking flies.

No effort was made to rid any of the calves of ticks, body lice, etc., therefore, if the normal calves which daily entered the fly-compartment with the infected calf contracted *Trypanosoma pecorum* infection whilst the control calf remained healthy, then the Tabanus under experiment was in all probability the transmitting agent. If the control calf also contracted the disease, then some agency other than Tabanidæ, such as ticks, etc., would probably be responsible. Lastly, if all the normal calves escaped infection, then presumably the Tabanus under experiment did not transmit the disease.

The Tabanidæ did not appear to live more than three or four days, in spite of the presence of running water, shrubs and foliage in the fly-compartment. The majority died within the first 48 hours. A few fed greedily on the calves for 12 to 18 hours after capture.

The normal calves and the control calf were examined bi-weekly for trypanosomes throughout the experiment, and for one month after its completion. The infected calf was also examined bi-weekly; *Trypanosoma pecorum* were always seen in its blood; sometimes they were present in large numbers, sometimes scanty.

Table I gives the result of these experiments at a glance.

Table I.

Species of Tabanus used.	Experiment No. of calves.	No. of days Tabanidæ fed on calves.	Approximate No. of flies used.	Result.
Tabanus secedens (Walk.)	2304 & 2305	30	1500	Negative; calves remained healthy.
Tabanus fuscomarginatus (Ricardo)	2400 & 2401	43	2150	" "
Tabanus thoracinus (Pal. de Beauv.)	2542	55	2650	" "

Remarks.—It is seen that the Tabanidæ had ample opportunity to feed indiscriminately on the infected and healthy calves over a considerable period of time. Large numbers of flies were used, yet no normal calf contracted the disease. The fact that the Tabanidæ died within 48 hours of being put in the kraal does away with the possibility of a development of *Trypanosoma pecorum* in the flies. These experiments, therefore, only prove that these flies do not transmit the trypanosome mechanically.

4. A STUDY OF THE FLAGELLATES FOUND IN TABANUS SECEDENS, TABANUS FUSCOMARGINATUS AND TABANUS THORACINUS.

In this investigation two series of dissections were made. In the first wild flies caught in the neighbourhood of the laboratory were dissected. In the second series dissections were made of wild flies caught in the neighbourhood and introduced into the fly-compartment of the kraal, where they were used in the transmission experiments detailed above.

As wild flies were used throughout, it was necessary to carefully study the flagellates which may infect these flies, in order that parasites natural to the flies, such as Crithidia or Herpetomonas, should not be mistaken for developmental forms of pathogenic trypanosomes in the fly.

It should be noted here that the cattle which grazed in the neighbourhood where the flies were caught were healthy during the period that the investigations were being carried out.

In the first series of dissections 138 *Tabanus secedens* and 49 *Tabanus thoracinus* were dissected. Of the *Tabanus secedens* 7 (5 per cent.) showed a heavy infection of flagellates in the hind-gut, rectum, and proctodæum. The mouth parts and remainder of the alimentary tract contained no flagellates. Of the *Tabanus thoracinus* dissected, flagellates were found in the hind-gut, rectum and proctodæum of 13 (26·5 per cent.). The mouth parts and remainder of the gut were negative for flagellates. No *Tabanus fuscomarginatus* were dissected in this series.

The flagellates found in the hindermost portions of the intestines of both these species were similar. Two main types predominated.

Type 1, *Oval, Flagellated Parasites.*

A. *Living, Unstained.*

This type is very active, and darts rapidly across the field when free. More commonly large masses of these flagellates are seen to be irregularly grouped together, the flagella of the outermost ones rapidly vibrating. This type divides by longitudinal fission, and forms undergoing this process are commonly seen. Bright points can often be made out in the body-substance of these parasites (chromatin granules); no definite vacuoles could be seen. Masses of these flagellates sometimes occupied a whole field.

B. *Fixed and Stained.*

These flagellates contain a distinct macro- and micro-nucleus. The macro-nucleus is large, roughly circular and sometimes diffused. It lies about the centre of the body of the parasite, often occupying its whole width. The micro-nucleus is circular or rod-shaped, is placed on the flagellar side of and close to the macro-nucleus. The flagellum arises from the micro-nucleus, it is short and projects from the body of the parasite, the protoplasm of the body being often prolonged for a short distance along the flagellum. There is no undulating membrane. Chromatin granules, irregular in number and size, are often to be seen in the vicinity of the macro-nucleus (Plate 16, figs. 1 to 6).

Type 2, *Circular, Non-Flagellated Parasites.*

These parasites contained macro- and micro-nuclei. The drawing clearly indicates their structure (Plate 16, figs. 7 to 14). In the fresh preparations they were motionless.

In the second series of dissections of the Tabanidæ which had been liberated in the fly-compartment of the kraal, 50 *Tabanus secedens*, 24 *Tabanus thoracinus*, and 37 *Tabanus fuscomarginatus* were dissected. Of the 50 *Tabanus secedens*, 2 were infected with flagellates (4 per cent.). Of the 24 *Tabanus thoracinus*, 1 was infected (4 per cent.). No *Tabanus* *fuscomarginatus* contained parasites. The three infected flies all contained flagellates in the hind-gut, rectum, and proctodæum, which resembled Types 1 and 2 mentioned above.

Type 3, Elongated, Flagellated Parasites.

One fly, a *Tabanus secedens*, contained many flagellates, of a different variety, in the fore-gut, mid-gut, and fore-part of hind-gut. In the fresh preparation this third type of flagellate was motile, and possessed a poorly developed undulating membrane, which was thrown into short folds. The movements of the parasite were active, but not translatory to any great extent. The accompanying drawing (Plate 16, figs. 15 to 24) so clearly show the structure of this type in the stained state that further description is unnecessary.

Type 3 occurs principally in the thick-walled mid-gut of the fly and, to a lesser extent, in the fore-gut and fore-part of the hind-gut. Types 1 and 2 occur only in the hind-gut, rectum, and proctodæum of the fly.

Inoculation Experiments carried out with these Parasites.

Expt. 2404.

April 23, 1910.—White rat inoculated, intra-peritoneally, with contents of hind-gut, rectum, and proctodæum of a wild *Tabanus secedens*. This fly had been in fly-compartment of kraal; it was heavily infected with Type 1 of the parasite.

Result.—Negative. Rat examined for one month after injection.

Expt. 2405.

April 23, 1910.—White rat inoculated, intra-peritoneally, with contents of fore-gut and mid-gut of a wild *Tabanus secedens*. This fly had been in kraal, and its fore-gut and mid-gut were swarming with Type 3 of the parasite in an active state.

Result.—Negative. Rat examined for one month after the injection.

Expt. 2411.

April 27, 1910.—White rat inoculated, intra-peritoneally, with hind-gut, rectum, and proctodæum of a wild *Tabanus secedens*. This fly had never been in kraal. It was heavily infected with Type 2 of the parasite.

Result.—Negative. Rat examined for one month after injection.

Expt. 2412.

April 27, 1910.—White rat inoculated, intra-peritoneally, with hind-gut, rectum, and proctodæum of a wild *Tabanus secedens*. This fly had never been in kraal; it was heavily infected with Type 1 of the parasite.

Result.—Negative. Rat examined for one month after injection.

Expt. 2412B.

July 4, 1910.—White rat inoculated, intra-peritoneally, with the pooled citrated contents of the hind-gut, rectum, and proctodæum of two wild *Tabanus thoracinus*. These flies had never been in kraal and were heavily infected with Types 1 and 2 of the parasite.

Result.—Negative. Rat examined for one month after injection.

CONCLUSIONS.

1. *Tabanus secedens*, *Tabanus thoracinus*, and *Tabanus fuscomarginatus* appeared to be unable to transmit *Trypanosoma pecorum* from infected to healthy cattle by the mechanical method of transmission.

2. Owing to the short life of these tabanids in captivity it is impossible, from the above experiments, to state whether they can convey the disease (*Trypanosoma pecorum*) after a period of development of the trypanosome in the fly.

3. We believe the three types of flagellates found in *Tabanus secedens* and *Tabanus thoracinus* to be various stages in the development of a harmless Crithidium in these flies.

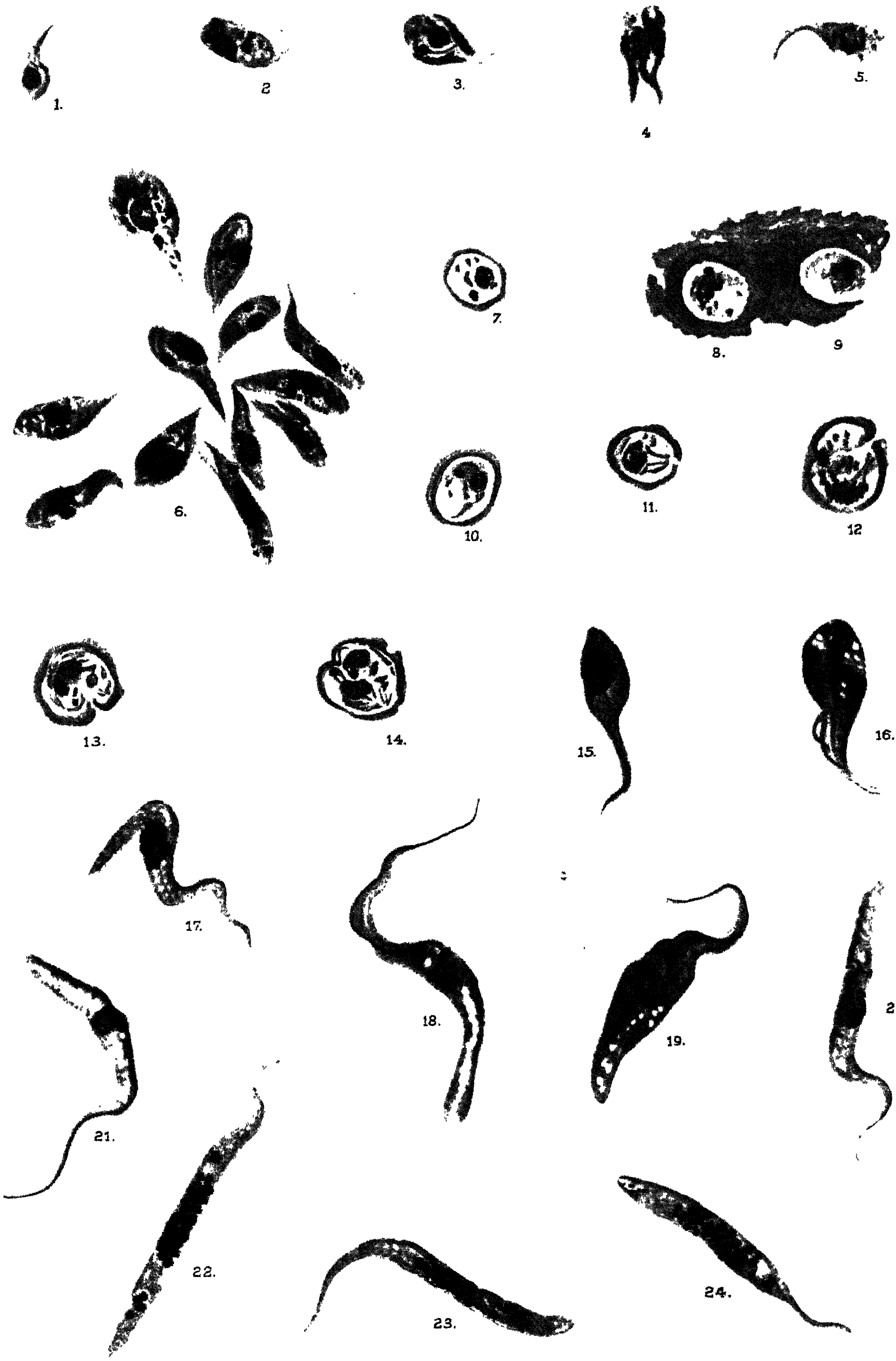
DESCRIPTION OF PLATE 16.

Smear preparation of hind-gut, rectum and proctodæum of a *Tabanus secedens*, stained Giemsa, × 2000.

Figs. 1—6, Type 1, oval, flagellated parasites. Fig. 4 is a dividing form of this type. Fig. 1 shows the most common form of this type. Fig. 6 represents an irregular mass of the parasites; these masses often contain very large numbers of the parasites. The flagella are poorly stained in these preparations, and so do not show up well (Expt. 2412).

Figs. 7—14, Type 2, circular, non-flagellated parasites. Made from smear preparations of the rectum and proctodæum of a *Tabanus secedens* (Expt. 2411).

Figs. 15—24, Type 3, elongated, flagellated parasites. Figs. 17 and 18 show the most typical forms of this type. Fig. 19 is a stout atypical form, which was not common. Fig. 15 represents a form which would appear to be reverting to Type 1. Fig. 16 is a dividing form. The parasites figured in Figs. 15—24 were obtained from a smear made of the mid-gut of a wild *Tabanus secedens* which had been in the fly-compartment of the cattle kraal (Expt. 2405).



M. E. Bruce, del.

Complement Deviation in Mouse Carcinoma.

By J. O. Wakelin Barratt, M.D., D.Sc. Lond., Director of Cancer Research Laboratory, University of Liverpool (Mrs. Sutton Timmis Memorial).

(Communicated by Prof. C. S. Sherrington, F.R.S.,—Received December 5, 1910,—Read February 2, 1911.)

A characteristic feature of malignant growths is the occurrence of degeneration, usually in the more central portions of the growth. It is therefore possible that antibodies might make their appearance in the blood plasma in respect of the dead material thus set free. The presence of such antibodies could be sought for in several ways, for example, by observing if any of the insoluble constituents of the tumour could be agglutinated or caused to dissolve, or any of the soluble constituents obtainable from the growth could be precipitated, by the blood serum of the host. There is, however, another way of testing for the production of such antibodies, namely, by observing if the serum when mixed with the tumour, or with a watery or alcoholic extract of the tumour, can fix complement. In this case the method followed is an application of the observation of Bordet,* that an immune serum contains a thermostable substance (amboceptor or immune body), which when mixed with the antigen which has been employed to determine its production is capable of absorbing complement. So far as I can ascertain from a search of the literature of complement deviation, no investigation on these lines has, up to the present, been made in connection with malignant growths.

It was, therefore, decided to test for the presence of antibodies in respect of malignant growths, using in the first instance the method of complement fixation. For the purpose of investigation carcinoma of the mouse appeared exceedingly suitable, since it affords a condition which is readily producible and represents a single pathological entity, the same strain of tumour being inoculable from animal to animal so long as may be desired. Only under such circumstances is it possible to make a comparative series of observations under conditions of experiment which are throughout identical in respect of the tumour employed.

These experiments fall into two groups: those in which complement of the rabbit or of man was employed; those in which the complement of the mouse was used.

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* "Les Sérums Hémolytiques," 'Ann. de l'Inst. Pasteur,' 1900, vol. 14, p. 257.

Experiments in which the Complement Employed was that Present in the Serum of the Rabbit or of Man.

Method.—The hæmolytic system selected consisted (1) of the red blood cells of the rabbit, employed in a 2½-per-cent. suspension in 0·85-per-cent. sodium chloride solution; (2) of immune body derived from the serum of the rat; and (3) of complement contained in human blood serum or that of the rabbit. The rat providing the immune serum had been previously inoculated intraperitoneally on three occasions at intervals of 10 to 14 days, receiving each time about 0·3 gramme of the red blood cells of the rabbit, previously washed in 0·85-per-cent. solution of sodium chloride. Before being used the serum was inactivated by heating to 57° C. for one hour. The hæmolytic power of the serum of the rat varied with different animals; that used in the experiments recorded in the tables was capable of completely hæmolysing, in one and a-half hours, at a temperature of 37° C., four to five times its volume of the red blood suspension employed. In sensibilising the red blood cells of the rabbit one part of serum was added to one part of the red blood cell suspension, and the mixture allowed to stand at 37° C. with occasional shaking, for one hour and a-half. A slight degree of agglutination of the red blood cells took place on the addition of the inactivated serum of the rat, but this did not give rise to any difficulty. A further slight increase in the degree of agglutination occurred when human blood serum was subsequently added; this did not occur, however, if the blood serum of the rabbit was employed as the source of complement, for which reason in most of the experiments the latter was used. The amount of serum obtained from the rat varied from 0·5 c.c. to 2 c.c.; on keeping this serum a precipitate of proteid sometimes slowly separated out.

The above hæmolytic system was chosen chiefly because the animals employed in its preparation are readily obtainable and can be kept in a laboratory.

As antigen, an alcoholic extract of mouse tumour was prepared by adding 90 c.c. of absolute alcohol to 19 grm. of tumour. The procedure adopted was as follows:—The tumour was placed in a mortar, together with the alcohol, and was then converted into as fine a pulp as possible. After standing at room temperature for six days, with occasional shaking, the alcoholic liquid was poured off and filtered. For use in the experiments recorded below, one part of the alcoholic extract was added to five parts of 0·85-per-cent. solution of sodium chloride. The alcoholic extract was floated on to the top of the solution of sodium chloride, and, after standing for about five minutes, admixture was completed by shaking the tube, when a very turbid liquid, free from precipitate, resulted.

Serum was obtained from mice affected with mouse tumour by killing these animals with ether, exposing the heart with antiseptic precautions, puncturing the right ventricle with a sterilised glass pipette provided with a capillary point which was pushed into the right auricle. With the aid of a rubber teat blood was sucked into the pipette and transferred to a sterilised centrifugal tube, in which it was allowed to coagulate. The latter was then centrifugalised, the supernatant serum pipetted off, and inactivated by heat as above described. The amount of serum obtained from the mouse ranged from 0·1 c.c. to 0·3 c.c. For use in control experiments, serum was obtained by the same procedure from normal mice. The sera were not employed for experiment until the lapse of 24 hours after inactivation.

Owing to the small amount of serum obtainable from the mouse, it was necessary to deal with very small quantities of the fluids used for experiment. To this end capillary pipettes were prepared, which were graduated in hundredths of a c.c., and were capable of delivering amounts as small as 0·003 c.c. to 0·001 c.c. The graduation of the pipettes was effected by determining the diameter of the capillary portion with the aid of a microscope provided with an eyepiece micrometer. From this the sectional area of the capillary tube was determined, and the length corresponding to any desired number of cubic millimetres calculated. The tubes were so selected that the length corresponding to 0·01 c.c. was from 1 cm. to 2 cm. The mixtures of fluids employed in Tables I to IV were preserved in small tubes of about 5 mm. internal diameter.

The actual testing was carried out in the following way:—In the first place a number of tubes were prepared containing sensibilised red cells. To this end, into each tube 0·02 c.c. of the inactivated serum of a rat into which the red blood cells of the rabbit had been injected as above described was placed, and then 0·02 c.c. of the suspension of the red blood cells of the rabbit, the mixture being kept at 37° C., with occasional shaking, for one hour and a-half. At the end of this time the fluid part of the mixture was removed by means of a capillary pipette from the red cells which had been allowed to fall to the bottom of the tube.

The tubes containing sensibilised red cells were now arranged in four groups (*cf.* Tables I to IV). To the tubes in each group complement was added in amounts ranging from 0·001 c.c. to 0·081 c.c. To each of the tubes of the first group 0·06 c.c. of a 0·85-per-cent. sodium chloride solution was added, so as to make the volume of fluid in each tube equal to that in the corresponding tubes of the other groups. In this group the hæmolytic power of varying quantities of complement was exhibited. To each of the tubes of the second group 0·03 c.c. of sodium chloride solution and 0·03 c.c. of tumour extract was added. This group exhibited the effect of tumour extract in fixing a relatively small amount of complement. Sodium chloride was replaced in the third group by inactivated normal serum of the mouse, and in the fourth group by inactivated serum obtained from a mouse with a tumour. In both the latter groups a further and more considerable fixation of complement took place, and a comparison of the two enabled the effect of mouse carcinoma upon complement fixation to be determined.

Experimental Results.—When a complement deviation test, as above described, is applied to the serum of a mouse inoculated with mouse tumour, a positive reaction is obtained in some cases, in others a negative reaction is observed.

The course of a positive reaction is exhibited in Tables I to IV, which will now be considered.

In Table I the action on sensibilised red blood cells of healthy human serum obtained a few hours before use is exhibited. An exceedingly slight degree of laking was produced by 0·001 c.c. of this serum, and it may be added that half this quantity produced no laking recognisable to the naked eye at the end of 90 minutes; 0·003 c.c. and larger amounts of human serum produced complete hæmolysis within this period of time. When active normal rabbit's serum was employed instead of human serum in the experiments given in this and the following tables, the degree of the resulting hæmolysis was slightly lowered.

Table I.—Hæmolytic Power of Complement contained in Human Blood Serum. The contents of each tube were mixed with sensibilised red cells obtained from 0·02 c.c. of a 2½-per-cent. suspension of the red cells of the rabbit, and kept, with occasional shaking, at 37° C. for 90 minutes.

No. of tube.	Active human blood serum.	0·85 per cent. solution of sodium chloride.	Degree of hæmolysis at the end of—			
			30 mins.	60 mins.	90 mins.	18 hours (at room temperature).
	c.c.	c.c.				
1	0·001	0·06	Not recognisable	Slight	Slight	Partial
2	0·003	0·06	Partial	Complete	Complete	Complete
3	0·009	0·06	Complete	"	"	"
4	0·027	0·06	"	"	"	"
5	0·081	0·06	"	"	"	"

It may here be pointed out that, although it is not difficult to recognise a pronounced degree of hæmolysis, nevertheless, owing to the smallness of the quantities dealt with, it is not possible to recognise a slight degree of laking, and, in particular, it is not possible to determine quantitatively the degree to which the red cells are laked, as is possible when large amounts of cells and fluid are available.

When tumour extract was added at 37° C. to human serum in the amounts given in Table II, and after the lapse of an hour and a-half the mixtures were transferred to tubes containing sensibilised red blood cells, laking took place slightly, but distinctly less readily, the first tube remaining free from laking, and the second tube presenting, instead of complete laking at the end of 90 minutes, only a slight degree of laking, which was slow in appearing. In the remaining tubes rapid and complete laking occurred. It follows, therefore, that the antigen is of itself capable of fixing a certain amount of the complement contained in the human serum employed.

Table II.—Complement Fixation by Mouse Tumour Extract. The contents of each tube, after standing at 37° C. for 1½ hours, were added to sensibilised red cells obtained from 0·02 c.c. of a 2½-per-cent. suspension of the red cells of the rabbit, and the mixture kept, with occasional shaking, at 37° C. for a further period of 1½ hours.

No. of tube.	Active human blood serum.	0·85 per cent. solution of sodium chloride.	Antigen (tumour extract).	Degree of hæmolysis at the end of—			
				30 mins.	60 mins.	90 mins.	18 hours (at room temperature).
	c.c.	c.c.	c.c.				
1	0·001	0·03	0·03	Not recognisable	Not recognisable	Not recognisable	Not recognisable
2	0·003	0·03	0·03	,,	Slight	Slight	Slight
3	0·009	0·03	0·03	Slight	Nearly complete	Complete	Complete
4	0·027	0·03	0·03	Partial	Complete	,,	,,
5	0·081	0·03	0·03	Nearly complete	,,	,,	,,

When, in addition to tumour extract, 0·03 c.c. of inactivated serum obtained from a normal mouse was added to the quantities of human serum given in Table III, and, after being kept at 37° C. for an hour and a-half, the mixtures were transferred to tubes containing sensibilised red blood cells, it was found that the action of the complement was further reduced, no hæmolysis occurring in the second tube, and the amount in the third tube being considerably diminished.

If, instead of employing normal mouse serum, the serum of a mouse with a large tumour was employed, the course of a positive reaction was such as is exhibited in Table IV, that is to say, a still further reduction in the amount of the hæmolysis took place,* no laking being observed at the end of

Table III.—Complement Fixation by Mouse Tumour Extract in the presence of inactivated normal Mouse Serum. The contents of each tube, after standing at 37° C. for 1½ hours, were added to sensibilised red cells obtained from 0·02 c.c. of a 2½-per-cent. suspension of the red cells of the rabbit, and the mixture kept, with occasional shaking, at 37° C. for a further period of 1½ hours.

No. of tube.	Active human blood serum.	Inactivated normal serum of mouse.	Antigen (tumour extract).	Degree of hæmolysis at the end of—			
				30 mins.	60 mins.	90 mins.	18 hours (at room temperature).
	c.c.	c.c.	c.c.				
1	0·001	0·03	0·03	Not recognisable	Not recognisable	Not recognisable	Not recognisable
2	0·003	0·03	0·03	"	"	"	"
3	0·009	0·03	0·03	"	Slight	Slight	Slight
4	0·027	0·03	0·03	Partial	Complete	Complete	Complete
5	0·081	0·03	0·03	Complete	"	"	"

Table IV.—Complement Fixation by Mouse Tumour Extract in presence of inactivated Serum of Mouse with Tumour. The contents of each tube, after standing at 37° C. for 1½ hours, were added to sensibilised red cells obtained from 0·02 c.c. of a 2½-per-cent. suspension of the red cells of the rabbit, and the mixture kept, with occcasional shaking, at 37° C. for a further period of 1½ hours.

No. of tube.	Active human blood serum.	Inactivated serum of mouse with tumour.	Antigen (tumour extract).	Degree of hæmolysis at the end of—			
				30 mins.	60 mins.	90 mins.	18 hours (at room temperature).
	c.c.	c.c.	c.c.				
1	0·001	0·03	0·03	Not recognisable	Not recognisable	Not recognisable	Not recognisable
2	0·003	0·03	0·03	"	"	"	"
3	0·009	0·03	0·03	"	"	"	"
4	0·027	0·03	0·03	"	Slight	Nearly complete	Complete
5	0·081	0·03	0·03	Nearly complete	Complete	Complete	"

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* In each comparative test the two series represented in Tables III and IV were performed simultaneously, the same active normal serum being used in both series.

90 minutes in the third tube, while the rate and degree of hæmolysis occurring in the fourth tube were also reduced, and in the fifth tube hæmolysis, though not prevented, was retarded. It must also be observed that the actual increase of complement in passing from the third to the fourth tube is considerably greater than that between the second and third tubes, owing to the circumstance that the quantities employed have been selected so as to form a geometrical progression, the amount of complement contained in each tube being three times that contained in the preceding tube. By adding equal increments of complement to the tubes, the difference between the last two series becomes more obvious, but as the number of tubes made up cannot be conveniently increased, it is necessary to reduce the range over which the investigation extends. In this, as in the previous series, no considerable change occurred on keeping the tubes at room temperature (14° C. to 17° C.) for a further period of 18 hours. In making the last two series of experiments, recorded in Tables III and IV, serum was obtained on the preceding day from a normal mouse and from a mouse with a tumour; both sera were sterilised at the same time, and were kept, as already mentioned, for 24 hours before use.

Hæmolysis in these experiments proceeds slowly. The quantities dealt with being very small, it is not possible to attempt to discover the formula representing the reaction velocity or to ascertain the end equilibrium. It is, however, clear that it is not at present permissible to treat the reaction as a stoichiometric one, and to represent in terms of red blood cells the quantities of complement fixed. This is well exhibited in Table IV, Experiment 5, in which it is seen that the reaction rate is retarded, though if the reaction were simply stoichiometric this should not occur, owing to the much greater amount of complement in tube 5 as compared with tube 4. A better means of comparison would presumably be afforded by the determination of the reaction constants.

In the observations recorded above, it was found, on comparing the third and fourth series (Tables III and IV), that the degree of hæmolysis occurring when serum obtained from a mouse with a tumour was employed was less in degree, and proceeded more slowly, than when the serum of a healthy mouse was used. In other words, during the period of experiment, more complement was fixed with the former serum than with the latter. This result was not, however, always observed. Sometimes the rate and degree of hæmolysis were not appreciably different in the two series; the weight of the tumour of the mouse furnishing the former serum in these cases amounted to from about one-sixth to one-quarter or more of the total body weight. The number of experiments so far made does not furnish many data bearing upon the cause of this difference in respect of complement fixation. It would be expected that the degree of fixation would be likely to be marked when the tumour was considerably degenerated. In the earlier experiments the condition of the tumour in this respect was not noted. In the last five experiments, however, attention was paid to the condition of the tumour, and deviation was found to be increased when marked degeneration was present (two cases), but was unaltered when degeneration was slight in rapidly growing tumours (three cases).

Experiments in which the Complement employed was that Present in the Serum of the Mouse.

Exception may be taken to the experiments already described on the ground that there is no direct proof that the complement employed fits the amboceptor of the autotoxin for which search has been made. There is, however, indirect proof that this is so, since the complement employed is fixed by the amboceptor in the presence of antigen contained in the tumour extract. As, however, the degree of complement fixation observed in the preceding experiments was slight, it was decided, in view of the importance of the issues involved, to repeat these experiments in a form in which this objection was avoided, namely, by employing as complement that present in the normal serum of the mouse.

Method.—The lines along which this investigation proceeded are as follows: The first stage was to discover a suitable source of red blood cells, preferably one for which the serum of the mouse normally possessed a hæmolysin. The action of the normal serum of the mouse upon several easily obtainable varieties of red blood cells was tested, with the results given in Table V. The red cells employed were those of the rabbit, guinea-pig, rat, man, and goat. It will be seen that the degree of hæmolysis obtainable with these red cells was much less than that ordinarily occurring when a hæmolytic serum prepared by injecting red cells intraperitoneally is employed. When the different varieties of red cells are compared it is seen that those of the rabbit were hæmolysed far more readily than any of the other forms employed. The red blood cells of the rabbit were therefore selected as the most convenient to employ for testing for deviation of the complement of the mouse.

The hæmolysin in question in normal serum of the mouse is present in relatively small amount. Subsequent observations showed that the amount normally present was occasionally considerably less than that indicated in Table V.

Table V.—Hæmolytic Action of Mouse Serum upon Red Blood Cells derived from various Sources. Temperature of experiment, 37° C.

No. of tube.	2½ per cent. suspension of red blood cells.	Active serum of mouse.	Hæmolysis at the end of 90 minutes.				
			Red cells of man.	Red cells of goat.	Red cells of rabbit.	Red cells of guinea-pig.	Red cells of rat.
	c.c.	c.c.					
1	0·02	0·001	Not recognisable	Not recognisable	Partial	Not recognisable	Not recognisable
2	0·02	0·003	Exceedingly slight	,,	Nearly complete	,,	Exceedingly slight
3	0·02	0·009	,,	Exceedingly slight	Complete	Exceedingly slight	,,
4	0·02	0·027	,,	,,	,,	,,	,,
5	0·02	0·054	Slight	Slight	,,	Slight	Slight

It therefore became necessary to obtain a supply of mouse serum sufficiently rich in hæmolysin for use, after inactivation, in sensibilising the red blood cells of the rabbit. It was also necessary that the amount of serum obtained should be enough for an extended series of observations. To this end 23 mice received intraperitoneally injections of the red blood cells of the rabbit in the following amounts:—

0·005 c.c. of red cells on the 1st day.
0·01 ,, ,, 3rd ,,
0·02 ,, ,, 4th ,,

Nineteen of these mice still surviving were killed with ether on the 18th day, and immediately after death blood removed with aseptic precautions from the right ventricle of each by means of a glass pipette. The sera thus obtained were mixed together and the hæmolytic power of the mixture upon the red blood cells of the rabbit determined. The results obtained are given in Table VI. The maximum amount of serum required to completely hæmolyse, within 90 minutes, 0·02 c.c. of a 2½-per-cent. suspension of the red cells of the rabbit was found to be about 0·009 c.c. In sensibilising the red blood cells of the rabbit with this serum, after inactivation, when testing for complement in the experiments described below, twice this minimum amount was employed, the time allowed for sensibilising at 37° C. being one hour.

The method pursued in the further course of these experiments was so similar to that followed in the preceding section that no additional description of technique employed is required.

Experimental Results.—The action of fresh* mouse serum on the sensibilised red blood cells of the rabbit ranged, in these experiments, between that recorded in the sixth column in Table V and that given in Table VI. Experiments made with sera of normal mice containing smaller amounts of complement did not permit a sufficient range of action and, in consequence, were not available for complement deviation tests.

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* That is to say, obtained within 30 to 60 minutes of the death of the animal.

Table VI.—Hæmolytic Action upon Red Blood Cells of Rabbit of active Serum obtained from 18 Mice which had previously received Injections of Rabbit's Red Cells. Temperature of experiment, 37° C.

No. of tube.	2½ per cent. suspension of red blood cells of rabbit.	Active serum of inoculated mice.	Hæmolysis at the end of—	
			30 mins.	90 mins.
	c.c.	c.c.		
1	0·02	0·001	Not recognisable	Exceedingly slight
2	0·02	0·003	„	Slight
3	0·02	0·009	Nearly complete	Complete
4	0·02	0·027	„	„
5	0·02	0·054	„	„

The complement fixation power of the mouse tumour extract was tested with normal mouse serum as the source of complement. At first a series of observations was made in which varying amounts of tumour extract were mixed with a definite amount of normal mouse serum, and after keeping for one hour at 37° C. added to sensibilised red blood cells of the rabbit (the serum employed for sensibilising the red cells being pipetted off before adding the mixture of normal mouse serum and tumour extract). This series is given in Table VII. It will be seen that with normal mouse A 0·009 c.c. of tumour extract fixed the greater part of the complement contained in 0·01 c.c. of the normal active serum of the mouse. This was the usual result, but in the case of some mice, as B in the same table, the tumour extract fixed much less complement.

A second series of experiments, similar to the preceding but with the addition of varying amounts of inactivated normal mouse serum, was now made. The results are given in Table VIII. The amount of complement contained in 0·01 c.c. of active normal mouse serum was nearly completely fixed by 0·01 c.c. of mouse tumour extract in the presence of 0·01 c.c. of inactivated normal serum. This, it will be observed, does not differ markedly from the action of mouse tumour extract acting alone, exhibited in Table VII. If, however, the two series of experiments are carried out simultaneously, the same active mouse serum being employed in both, it is seen that greater complement fixation is obtained in the presence of inactivated normal mouse serum than when the tumour extract alone is employed.

Table VII.—Complement Fixation by Mouse Tumour Extract. The contents of each tube, after standing at 37° C. for 1 hour were added to sensibilised red cells obtained from 0·02 c.c. of a 2½-per-cent. suspension of the red cells of the rabbit, and the mixture kept, with occasional shaking, at 37° C. for a further period of 2 hours.

No. of tube.	Active serum of normal mouse.	0·85 per cent. solution of sodium chloride.	Mouse tumour extract.	Hæmolysis at end of—	
				30 mins.	120 mins.
			Normal Mouse A.		
	c.c.	c.c.	c.c.		
1	0·001	0·02	—	Not recognisable	Not recognisable
2	0·003	0·02	—	„	„
3	0·009	0·02	—	Partial	Almost complete
4	0·027	0·02	—	Complete	Complete
5	0·01	—	0·003	Exceedingly slight	Very slight
6	0·01	—	0·009	Not recognisable	Exceedingly slight
7	0·01	—	0·027	„	Not recognisable
8	0·01	—	0·054	„	„
			Normal Mouse B.		
9	0·003	0·02	—	Very slight	Marked
10	0·009	0·02	—	Slight	„
11	0·027	0·02	—	Nearly complete	Complete
12	0·054	0·02	—	„	„
13	0·01	0·02	0·003	Slight	Nearly complete
14	0·01	0·02	0·009	Exceedingly slight	Marked
15	0·05	0·02	0·003	Marked	Complete
16	0·05	0·02	0·009	Very marked	„

It is also necessary, for the purpose of deciding how much normal mouse serum should be employed in the experiments made to determine complement fixation (Table IX), to ascertain approximately the amount of amboceptor corresponding to the antigen contained in the mouse tumour extract. It can be seen from Table VIII that the amboceptor present in 0·01 c.c. of inactivated normal mouse serum,* plus that contained in 0·01 c.c. of active normal mouse serum, roughly represents the minimum amount of amboceptor corresponding to 0·01 c.c. of mouse tumour extract. The same relationship appears in Table IX, in which a further series of

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* The same inactivated normal mouse serum, consisting of the mixed sera obtained from a number of healthy mice, was used throughout the series of experiments shown in Tables VIII to X.

Table VIII.—Complement Fixation by Mouse Tumour Extract in the Presence of Inactivated Normal Mouse Serum. The contents of each tube, after standing at 37° C. for one hour, were added to sensibilised red cells obtained from 0·02 c.c. of a 2½-per-cent. suspension of the red cells of the rabbit, and the mixture kept, with occasional shaking, at 37° C. for a further period of two hours.

No. of tube.	Active serum of normal mouse.	0·85 per cent. solution of sodium chloride.	Inactivated serum of normal mouse.	Mouse tumour extract.	Hæmolysis at end of—	
					30 mins.	120 mins.
			Normal Mouse C.			
	c.c.	c.c.	c.c.	c.c.		
1	0·003	0·02	—	—	Slight	Marked
2	0·009	0·02	—	—	Marked	Nearly complete
3	0·027	0·02	—	—	Nearly complete	Complete
4	0·010	—	0·010	0·003	Slight	Slight
5	0·010	—	0·010	0·009	"	"
6	0·010	—	0·010	0·027	Very slight	Very slight
7	0·010	—	0·050	0·040	Not recognisable	Not recognisable
8	0·015	—	0·050	0·080	"	"
			Normal Mouse D.			
9	0·010	0·045	—	—	Partial	Nearly complete
10	0·020	0·035	—	—	"	"
11	0·030	0·025	—	—	Nearly complete	Complete
12	0·010	0·022	0·010	0·003	Exceedingly slight	Marked
13	0·010	0·015	0·010	0·010	"	Slight
14	0·010	—	0·010	0·025	Not recognisable	Not recognisable
15	0·020	0·012	0·020	0·003	Partial	Nearly complete
16	0·020	0·005	0·020	0·010	Not recognisable	Slight
17	0·020	—	0·020	0·025	"	Not recognisable
18	0·030	—	0·050	0·003	Slight	Partial
19	0·030	—	0·050	0·010	Not recognisable	Exceedingly slight
20	0·030	—	0·050	0·025	"	Not recognisable

observations is given. As in the experiments described in the preceding section, it is again not evident that a stoichiometric relationship exists between complement and amboceptor or between amboceptor and antigen.

In view of the above results it became evident that, in testing the relative amount of complement fixation effected by mouse tumour extract in the presence of inactivated mouse serum obtained respectively from normal mice and from mice with tumours, the most convenient amount of inactivated normal mouse serum (having regard to the small scale on which these experiments must of necessity be made) would be 0·005 c.c. to 0·01 c.c. and that 0·025 c.c. of tumour extract would contain much more antigen than would be required for the amboceptor (corresponding to the antigen) contained in this serum. The amount of inactivated tumour mouse serum to be employed would of course be the same as that of the inactivated normal mouse serum. The amount of active normal mouse serum employed as source of complement ranged from 0·02 c.c. to 0·06 c.c.

Table IX.—Complement Fixation by Amboceptor of inactivated Normal Mouse Serum in presence of an excess of Tumour Extract. The contents of each tube, after standing at 37° C. for 1 hour, were added to sensibilised red cells obtained from 0·02 c.c. of a 2½-per-cent. suspension of the red cells of the rabbit, and the mixture kept, with occasional shaking, at 37° C. for a further period of two hours.

No. of tube.	Active serum of normal mouse.	0·85 per cent. solution of sodium chloride.	Inactivated serum of normal mouse.	Mouse tumour extract.	Hæmolysis at end of—	
					30 mins.	120 mins.
	c.c.	c.c.	c.c.	c.c.		
1	0·010	0·075	—	—	Slight	Nearly complete
2	0·020	0·065	—	—	„	Complete
3	0·030	0·055	—	—	Partial	„
4	0·010	0·030	0·005	0·020	Not recognisable	Not recognisable
5	0·010	0·025	0·010	0·020	„	„
6	0·010	0·015	0·020	0·020	„	„
7	0·010	0·045	0·010	0·005	Not recognisable	Slight
8	0·010	0·040	0·010	0·010	„	Not recognisable
5	0·010	0·030	0·010	0·020	„	„
9	0·030	0·040	0·020	0·005	Very slight	Complete
10	0·030	0·035	0·020	0·010	„	Nearly complete
11	0·030	0·025	0·020	0·020	„	„

A number of experiments was now carried out, in which the degree of complement fixation by inactivated normal mouse serum was compared with that of inactivated tumour mouse serum in presence of an excess of antigen. In Table X, a series of observations of this character is given.

It is seen that when a comparison is made between the tubes in which inactivated tumour mouse serum was employed (7 to 9, 10 to 12, 13 to 15), and those in which inactivated normal mouse serum was used (4 to 6), the amount of complement fixation was distinctly greater in the former than in the latter. It is true that the difference in fixation is not profound, but it is nevertheless unmistakable. It must also be remembered that the amboceptor corresponding to the antigen is partly contained in the active normal serum employed, and that, in consequence of this, the difference between the two inactivated sera in respect of amboceptor is rendered less evident. The fact that a greater degree of complement fixation occurs

Table X.—Comparison of Complement Fixation in presence of Inactivated Serum obtained (*a*) from mice with tumours and (*b*) from a normal mouse. The contents of each tube after standing at 37° C. for 1 hour were added to sensibilised red cells obtained from 0·02 c.c. of a 2½-per-cent. suspension of the red cells of the rabbit and the mixture kept, with occasional shaking, at 37° C. for a further period of 2 hours.

No. of tube.	Active mixed serum of two normal mice.	0·85 per cent. solution of sodium chloride.	Inactivated serum of normal mouse.	Inactivated serum of mouse with tumour.	Mouse tumour extract.	Hæmolysis at end of—	
						30 mins.	120 mins.
	c.c.	c.c.	c.c.	c.c.	c.c.		
1	0·010	0·100	—	—	—	Slight	Slight
2	0·020	0·090	—	—	—	Marked	Nearly complete
3	0·030	0·080	—	—	—	Nearly complete	Complete
4	0·020	0·055	0·005	—	0·025	Very slight	Slight
5	0·040	0·035	0·005	—	0·025	Slight	Complete
6	0·060	0·015	0·005	—	0·025	Marked	„
			Tumour Mouse 1.				
7	0·020	0·055	—	0·005	0·025	Very slight	Very slight
8	0·040	0·035	—	0·005	0·025	„	Slight
9	0·060	0·015	—	0·005	0·025	Slight	Marked
			Tumour Mouse 2.				
10	0·020	0·055	—	0·005	0·025	Very slight	Slight
11	0·040	0·035	—	0·005	0·025	„	„
12	0·060	0·015	—	0·005	0·025	Slight	Marked
			Tumour Mouse 3.				
13	0·020	0·055	—	0·005	0·025	Not recognisable	Not recognisable
14	0·040	0·035	—	0·005	0·025	Very slight	Slight
15	0·060	0·015	—	0·005	0·025	Slight	Marked

when tumour mouse serum is employed indicates that this serum, in reality, contains a considerably greater amount of amboceptor corresponding to the antigen used than does normal serum. Experiments of the kind recorded in Table X were repeated with the same and with other tumour mice sera. Altogether, the sera obtained from six mice with well developed tumours were employed, and in each case two complement fixation tests were made. With one exception, all the tests gave a similar positive result such as is shown in Table X. The exception occurred when the observation made with the serum of Tumour Mouse 1 was repeated; in this case no clear difference could be observed between the tumour serum and normal serum. Below the actual and relative weights of the tumours present in the six mice used for these experiments are given.

No. of mouse.	No. of days after inoculation at which killed.	Weight of tumour.	Weight of tumour in percentage of total weight (*i.e.* body and tumour) of animal.
		grms.	Per cent.
1	31	2·0	10
2	31	0·5	5
3	31	0·5	5
4	31	1·0	10
5	69	4·0	24
6	18	3·0	10

Summary.

In the presence of mouse tumour extract, employed as antigen, the complement deviating power of the inactivated serum of mice with tumours was, in some cases, found to be greater than that of inactivated normal mouse serum; in other cases no difference between the two sera was recognisable.

Experimental Researches on Vegetable Assimilation and Respiration. VIII.*—*A New Method for Estimating the Gaseous Exchanges of Submerged Plants.*

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Introduction.

The method in universal use for evaluating assimilation in submerged plants consists in counting or measuring the evolution of bubbles of gas. This method gives satisfactory results in medium conditions, but fails at both extremes of the conditions—light, temperature, and CO_2-supply—which chiefly control the magnitude of assimilation. Critical work over a wide range of conditions is therefore impossible with it.

A really satisfactory method must take account of the alteration of the dissolved gases as well as of those that are liberated as gas-bubbles; because, when assimilation is slight, the oxygen formed may all dissolve in the water and no bubbles appear. When temperature is high the bubbles consist partly of other gases physically liberated from the water, and when the CO_2-content of the surrounding water is very high the bubbles escaping will consist chiefly of this gas.

Wishing to extend our researches from land-plants to water-plants, we have elaborated a method which is open to none of these objections, and which will be described in the present paper. In this method a continuous current of water containing dissolved CO_2 flows over the assimilating plant, and the difference in the CO_2-content of the water before and after contact with the plant is a measure of the assimilation taking place. Fewer bubbles are liberated under these conditions than when the water is stationary, but the gas given off is collected automatically, analysed and allowed for in estimating the total assimilation.

The plant is contained in a glass chamber, and the conditions of illumination and temperature and CO_2-supply are completely under control, so that the interaction of these different factors can be directly investigated.

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* The first six contributions of this series have appeared in the publications of the Royal Society; No. VII, by Miss A. Irving, on "The Beginning of Photosynthesis and the Development of Chlorophyll," will be found in the 'Annals of Botany,' October, 1910.

The results obtained by this method in a critical investigation of the relation between CO_2-supply and assimilation will occupy the next paper of this series.

Section I.—THE APPARATUS.

The apparatus is schematically represented in fig. 1. The current of CO_2-laden water flows by gravitation from the bottle A to the plant chamber B situated in the bath C; and, after passing up through it, thence by the route *d*, Y, *n*, *k*, into the lower end of one or other of the 200 c.c. pipettes D and E, finally escaping from them by the overflow tubes above into the measuring cylinders F or G.

The different parts of the apparatus call for description in some detail.

(a) *The Plant Chamber.*—The water-plant used is confined in a flat vertical glass-fronted chamber of oval outline, 18 cm. long and 11 cm. wide, the rim of which is formed by a wrought-iron frame 14 mm. square in section (for front view see inset to fig. 1). The iron rim is closed up to form a chamber by oval glass plates, back and front, which are luted firmly to it by a wax mixture of resin, beeswax, and vaseline. The back plate is waxed down permanently, but the front one is removed each time to put in material for a new experiment and then refastened.

The chamber contains a removable oval grid H (made of silver wire, with meshes about 6 mm. broad) which lies against the back of the chamber, and to this the plant material is tied down with thread. The grid, the iron frames, and all metallic parts in contact with the water current, are coated with wax to prevent chemical action and possible poisoning of the plant.* The iron frame of the chamber is pierced by an inlet tube (*b*) at its lowest point, and by an outlet tube (*d*) at its highest point. Close to the latter is a hole for a thermometer by which the internal temperature is determined. Immediately above the inlet tube is a thin sieve-like "baffle-plate," which stretches right across the opening and causes the inflowing water to spread out in all directions, hindering it from rising straight up the middle of the chamber.

(b) *The Water-Current.*—The effective fall which determines the rate of flow of the water-current through the apparatus is the difference in level between the mouth of the central tube of the Mariotte's bottle A and the mouth of the upper ends of the pipettes D and E where the water overflows.

It will become obvious that a uniform rate of flow is of fundamental importance in this method of investigation; the rate aimed at was about 300 c.c. per hour. The actual rate was continually determined by measuring

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* All parts are of silver or iron; no copper or brass is present.

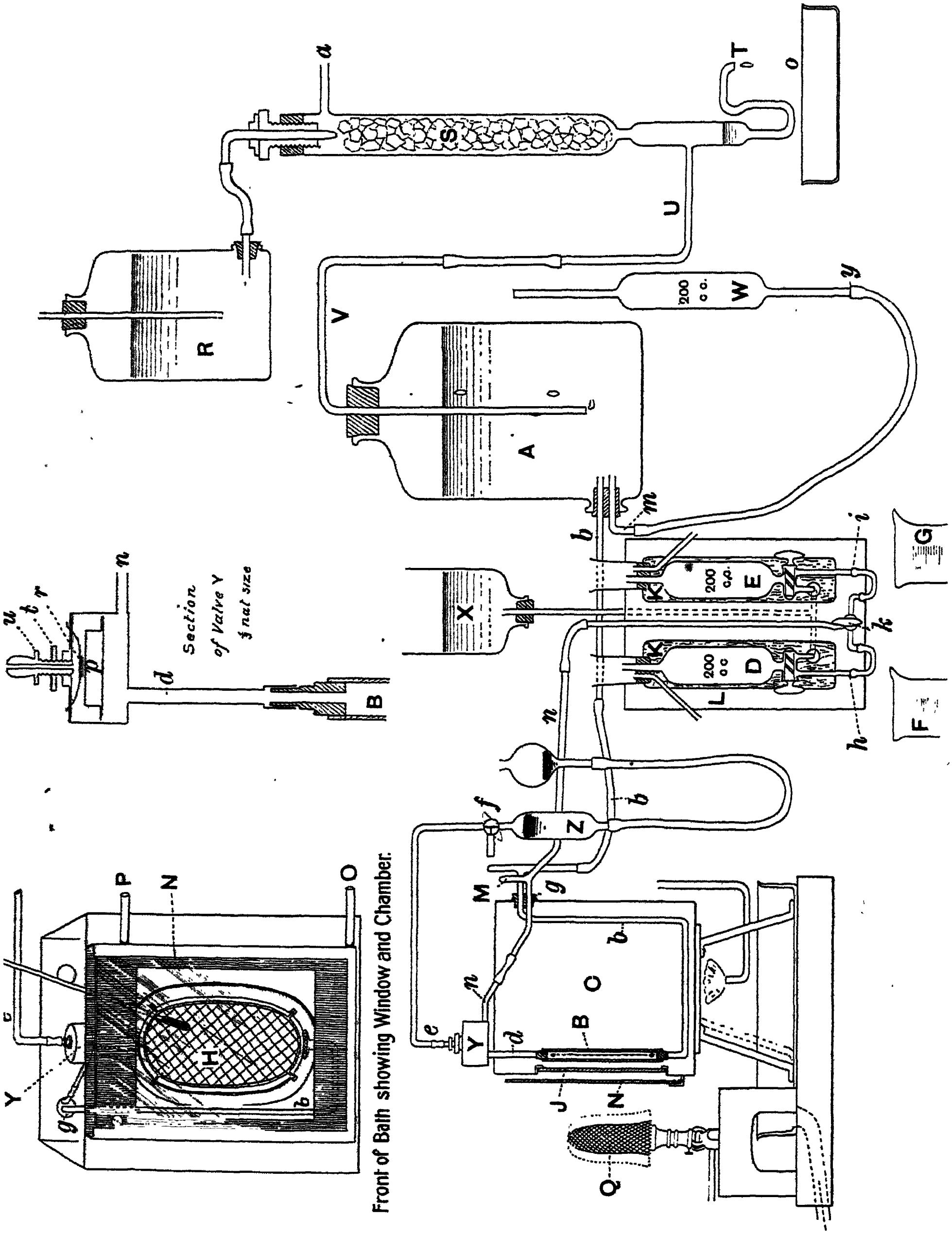

Fig. 1.

the amount overflowing from the pipettes into the graduated cylinders F and G. Any significant departure from uniformity was corrected by raising or lowering the pipettes, by sliding up or down on the board L the slips of wood K to which the pipettes are attached.

Most of the disturbances in the rate of flow were due to the liberation of tiny bubbles from the liquid in the effluent tube (*n*). The impediment to flow which they caused was removed by shaking the tube and working the bubbles up into the blind arm M.*

(c) *The Bath and Temperature Regulation.*—The temperature of the cubical copper bath of water in which the chamber was entirely immersed was kept constant by a thermostat when temperatures above that of the laboratory were wanted. (Only the burner below the bath is shown in the figure.) Temperatures between those of the laboratory and the water supply could be kept constant merely by a regulated circulation of water through the bath. The lowest temperatures used were obtained by careful regular additions of small quantities of ice.

In the front of the bath is a large glass window J for lighting the assimilation chamber, and in front of the window a screen of rapidly-circulating cold water to cut off the heat of the illuminating burner Q from the bath. This screen is made by a rectangular brass frame fastened to the front of the bath and carrying a second glass plate N. The space between J and N is full of water; O is the inflow and P the outflow for this water screen.†

(d) *Light for Assimilation and its Regulation.*—All the experiments were carried out with a special artificial light, that of the triple Keith high-pressure burner used in this laboratory since 1903. The burner Q was placed on a small wooden table fixed in front of the bath. The intensity of the light is determined by its distance from the assimilating plants. When the fronts of the nearest pair of mantles are 130 mm. from the front of the assimilation chamber, the arbitrary value of 6 has been assigned to the light-intensity.‡ From this standard the intensity in other positions can be calculated.

(e) *The Supply of CO_2 Solution.*—Tap-water that had been boiled for some time to remove the CO_2 and the calcium carbonate was used as being more wholesome for the plants than distilled water. A stock of this was saturated with CO_2 which had been carefully scrubbed free from HCl, and diluted with more boiled tap-water to obtain the various strengths needed. Water cannot be saturated with CO_2 by merely bubbling the gas through it, even in a very long time; prolonged violent shaking of the water with a large volume of pure CO_2 will, however, achieve this.

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* There is a small omission in fig. 1. The tube rising vertically from *k* should be continued up to a free end closed by a tap, used for sucking out obstructing bubbles, and the tube *n* should be attached to a lateral branch below the tap. Further the part of tube *b*, *b* represented as rubber should really be all glass.

† The screen carries about 1 inch thickness of water, and the front glass is not cemented in watertight, but pressed firmly against the metal rim all round by corks between it and the bath. It is therefore easy to replace if cracked, and the very moderate leakage trickles into the trough below the bath and is carried away at I.

‡ See Matthaei, 'Phil. Trans.,' B, 1904, p. 59.

The properly diluted CO_2-solution is filled into the bottle A whence it can flow to the chamber B by the tube *b*. As the solution flows out of the bottle air is drawn in down the central tube V, and on the principle of "Mariotte's bottle," the rate of outflow is constant and independent of the level of the liquid in the bottle.

If laboratory air were to be drawn in this way into the bottle it would carry some of the CO_2 out of solution with it to the top of the bottle and the liquid would get progressively weaker. To prevent this the incoming air is charged with just as much CO_2 as should be in equilibrium with the solution of CO_2 in the bottle, and alteration of the strength is thereby prevented. This addition of CO_2 to the air-current is accomplished by making the air traverse a CO_2-generating tower S, on its way to the bottle.

Hydrochloric acid of determined strength drops regularly from R on to the marble in this tower, trickles down and flows away below as a neutral liquid at T. The air entering above at (*a*) carries away the CO_2 generated and is thus enriched to the desired amount before it leaves by the tube U. Minor adjustment of the amount of CO_2 generated may be made during the course of an experiment by altering the rate of flow of the HCl by raising or lowering the dropper of the acid bottle by means of a screw attachment at the top of S.

The strength of acid required for any experiment was determined empirically and it was found that this whole procedure was quite satisfactory for maintaining constancy of CO_2-content in the water flowing into the assimilation chamber. The constancy attained in most experiments is shown in the successive analyses of samples withdrawn at *m* at regular intervals of time.

(f) *Estimation of the CO_2 in the Solutions.*—The fundamental proceeding of the whole method is the determination of the amount of dissolved CO_2 in (1) a sample of the liquid flowing from A to the assimilating plant in its chamber B and in (2) a sample of the effluent in D or E after it has passed over the plant. The samples analysed were always 200 c.c. of the liquid. For (1) a sample was drawn from the supply bottle by the tube *m* into a 200 c.c. pipette W at any given time and for (2) the 200 c.c. of liquid accumulated in pipette D or pipette E through which the effluent had been flowing were taken. This liquid can be drawn out by switching the current into the other pipette by the three-way tap *k* and then disconnecting the rubber joint at *h* or *i*.

Above the analytical bench on a high shelf are set two 30-litre bottles containing N/10 standard baryta and N/20 HCl* having burettes in connection with them, one to the baryta, and two to the acid for two simultaneous analyses. The procedure for estimating the dissolved CO_2 is to introduce 25 or 50 c.c. of the standard baryta into a clean glass cylinder of about 500 c.c. capacity and to take this to the apparatus and draw off into it the 200 c.c. of CO_2-solution. The cylinder is brought back and corked up in air-tight connection with one of the burettes of N/20 HCl and the excess of free baryta titrated by this acid, using phenolphthalein as indicator and stirring by means of a current of CO_2-free air forced through the liquid. The titre of the original 25 or 50 c.c. of baryta being known, the CO_2-content of the 200 c.c. of the liquid is obtained in equivalents of N/20 HCl, and the weight of CO_2 in 100 c.c. of liquid is calculated.

(g) *Method of Securing Uniform Flow of Effluent.*—In maintaining the regular flow of the current a special difficulty arises from the fact that the outflow pipettes are at intervals completely emptied of their liquid for analysis. Should the flow be switched back to an *empty* pipette when it is desired to analyse the contents of the other pipette then, of course, there would be a sudden rush of the current owing to the whole height of the pipette being added to the effective fall which governs the rate of flow. It is therefore essential that, when the circulation liquid has been withdrawn for analysis, the pipette be filled up with some liquid or other before the current can be safely turned back into it without disturbing acceleration of rate.

For this purpose there is a reservoir of liquid X from which either pipette can be quickly filled by turning its controlling tap. This liquid is water containing about 7 per cent. of alcohol and tinged with methylene blue. Its lower specific gravity allows this accessory liquid to float up in the pipette on top of the inflowing stream of the proper solution without mixing with it. The colour enables one to see that there is a sharp line of separation kept between the two liquids and also to follow the rise of the accessory liquid and tell when the whole of it has been driven out at the top of the pipette, and the contents again consist only of the proper effluent from the chamber suitable for making another analysis.

If the contents of each pipette were withdrawn for analysis as soon as the blue liquid was all driven over then none of the actual liquid from the chamber would ever pass away into F and G, but the whole of it could be analysed for its CO_2-content.

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* In later years N/5 baryta and N/10 HCl have been employed.

As the current rate is 300 c.c. per hour and each pipette holds 200 c.c., this would mean an analysis about every 40 minutes. In practice an analysis about every hour is thought sufficient, and for the other 20 minutes the current solution drops over into the measuring cylinders.

(h) *Analysis of Gas-bubbles liberated by the Plant.*—When the CO_2-content of the current through the assimilation chamber is low there may be an almost complete absence of "bubble-formation"; all the oxygen formed being carried off in solution in the liquid. With more active assimilation bubbles are liberated in the chamber; these are never pure oxygen, but contain always CO_2 in proportion to the CO_2-pressure in the liquid.

It follows that just as in strong CO_2, bubble-counting alone gives an exaggerated value of assimilation; so also analysis of the diminution of CO_2 in the liquid alone gives too great a value for assimilation, because some of the CO_2 gone from the solution has really diffused into the gas-bubbles that are forming. For the correct measure of assimilation this physical loss must be estimated and subtracted from the total apparent loss of CO_2.

To provide for this the gas-bubbles given off from the plant are separated from the liquid, continuously collected and analysed at appropriate times by means of the valve Y and the gas collector Z. The water-current leaving the top of the assimilation chamber by *d* passes immediately through a hollow metal drum Y on its way to the outflow tube *n*. This drum (see the large scale drawing of Y at the top of fig. 1) contains an outlet tube (*e*) in its roof, through which the gas that rises above the water, and the gas alone, is drawn off to the gas collecting vessel Z. Z is connected with a reservoir of mercury, which is lowered so that there is a strong suction exercised through *f*, *e* to the valved outlet in the top of the drum.

In the drum is the very light, loosely fitting, hollow metal float *p*. If the drum is full of water this float rises as high as possible and presses the little metal disc *r* (mobilely suspended by a strip of oiled silk) against the nozzle of the gas-outlet tube. This prevents any escape of water in the direction *e*, *f*, in spite of the suction from the mercury reservoir. When a certain amount of gas from the chamber has collected round the float in the top of the drum, the float sinks by its own weight, and the disc *r* drops and allows the gas to be sucked out through *e* till the rising water carries *p* and *r* up again to close the nozzle. The nozzle-tube *e* can be screwed down through the roof by the flange *u* till the valve mechanism is working well, and then locked in position by the screw collar *t*.*

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* The roof of the drum is not soldered in but fits tightly into a rabbet (not shown),

This form of valve has worked quite smoothly and the gas given off passes, with an occasional drop of water, intermittently into the receiver Z. From here by turning tap *f* and raising the mercury reservoir the gas can be driven through the side tube into a eudiometer for analysis.

From the shrinkage in volume of the gas after treatment with KHO in the eudiometer the absolute amount of gaseous CO_2 physically liberated from the water-current during the time since the last analysis is determined: this is used as a correction to obtain the proper assimilation.

Section II.—THE APPLICATION OF THE METHOD.

(a) *Procedure in a Typical Experiment.*—As an example of the method pursued in carrying out an experiment, and the calculation involved in arriving at the real assimilation, we propose now to describe a single case in detail. We select the first experiment done in 1907 when the apparatus had taken on the final form just described. The object of this experiment was to determine the assimilation by Elodea with an illumination equal to 5·7, at a temperature about 20° C., and a CO_2-supply of about 0·03 grm. per cent. (about one-sixth saturated).

The Elodea was gathered at 9.45 A.M. on June 7th in a stream about a mile from the laboratory, brought back in a tin and placed at once in tap-water. The first proceeding is to set up the material in the assimilation chamber.* Only the greenest and healthiest shoots were selected from the gathering, and these were laid down one by one on the silver grid until a continuous dense green layer, without any gaps, was formed on the front side of the grid. The whole was secured with cotton, the grid slipped into the chamber (its four projecting pegs fitting into slots in the iron frame), and the front glass, which had been carefully cleaned since the previous experiment, was again waxed on.

To accomplish this the glass was pressed down on to the film of wax left on the metal frame, and then lumps of wax-mixture were placed round the edge at intervals. These were melted and run uniformly round with a tiny gas-flame from a capillary tube at the end of a length of flexible tubing, the

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the junction being waxed over watertight. The drum can, therefore, be easily opened for inspection and adjustment. The strip of oil silk emerges through fine slits in the roof, which are also closed by wax.

* At the end of each experiment the chamber is opened by taking off the front glass, wiped and left to dry; the silver grid is removed, and the plant carefully taken off and spread out to dry up quickly in the air in order to obtain its dry weight. The bent glass inlet tube below, *b*, and the valve-drum Y above are never detached from the chamber. The widened lower end of the tube of the drum just slides over the metal outlet tube of the chamber, and so makes a rigid joint which indiarubber tube renders watertight (see large scale drawing at top of fig. 1).

chamber being kept horizontal. It may take some minutes to make a completely air-tight joint all round, and the application of the gas-flame may warm the chamber up a few degrees, but as there is a thermometer inside, it can be seen that the temperature reached is not injurious. During this time there is no water inside and, before admitting water, the chamber is tested for leaks by a gauge.

The chamber is then adjusted upon a tall brass stand (not shown in the figures, but similar to the one figured in "Assim. and Resp.," III, p. 57), and the whole lowered into the water-bath, previously regulated to the desired temperature. The position of the stand is shifted till the front of the chamber is 4 cm. behind the glass window of the bath, and the appropriate connections made at (*e*), (*n*), and (*g*).

The chamber is now to be filled up* with the proper CO_2-containing liquid which has been previously made and transferred into the supply bottle A. This is done by disconnecting the outlet tube *e* just above the valve Y and sucking till the solution first fills the chamber and then the valve and tubes. As soon as the tap *k* is turned to one of the pipettes D or E, the flow will commence. In this particular experiment this was done at 11.19 A.M., and pipette D (previously filled up with blue liquid) then began to overflow above at a steady rate, the overflow liquid dropping into the litre cylinder F. Illumination of the plant was begun at 11.25 by lighting the triple high-pressure burner (placed 13·4 cm. from the front of the chamber), and the circulation of water through the water-screen in front of the bath was started at the same time.

Everything has now been arranged, and the experiment starts its course at 11.25, see Table I on p. 383. It is obvious that, with a slow flow through the chamber, it will be some time before the contents of the pipette D represent the full effect of assimilation in removing CO_2 from the liquid. With rates about 300 c.c. per hour, the current must flow for more than an hour before it is worth while analysing the effluent.

In this experiment, as the table shows, the first sample of the effluent was withdrawn at 12.45, the current being then switched through pipette E already full of blue liquid. The CO_2 left in the 200 c.c. of liquid from D was still equal to 22·18 c.c. N/10 HCl, and comparison with the later numbers in the column shows that the full effect had not then been reached. The experiment was continued till 7 P.M., and six more analyses of the effluent were made, about one every hour, each representing the liquid flowing in 40 minutes. About once an hour also an analysis was made of the *affluent*

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* Before doing this, the dropping of acid on to the marble in the tower is started and adjusted to the appropriate rate known from previous experience.

Table I.—Experiment 1, June 7, 1907. *Elodea canadensis*, Dry Weight = 1·955 grm. Temp. 20°·3 C. Light at 13·4 cm., *i.e.* (L.I. = 5·7).

CO_2-content of 200 c.c. of current solution, expressed in c.c. N/10 HCl-equivalent.			Current rate, by outflow into measuring cylinders.			Temperature.			CO_2 carried off in gas-bubbles.
Time.	Affluent.	Effluent.	Time.	F.	G.	Time.	Bath.	Chamber.	
							° C.	° C.	
11.19 A.M. current started—	—	—	11.19			11.45	19·8	20·3	11.25 A.M. to 3.36 P.M., 4·8 c.c. CO_2 = 1·2 c.c. per hour.
11.44—11.46	[25·09]			[464 (324)]		12.4	20·0	20·5	
12.5—12.45		[22·18]D	12.45						
12.43—12.45	26·31				303	12.21	19·6	20·4	
1.2—1.42		19·45 E	1.42		(319)	1.5	19·0	19·8	
1.40—1.42	26·14			461		1.40	20·0	19·7	
1.46—3.6		19·04 D	3.6	(329)		1.55	19·8	20·5	—
3.4—3.6	25·59				310	3.6	19·8	20·4	
3.49—4.9		19·14 E	4.9		(295)	3.58	19·6	20·3	
4.7—4.9	25·36			306					3.36 P.M. to 6.0 P.M., 3·6 c.c. CO_2 = 1·5 c.c. per hour.
4.46—5.6		19·22 D	5.6	(322)		4.28	19·5	20·2	
5.4—5.6	25·98				306	5.27	19·8	20·4	
5.46—6.6		18·58 E	6.6		(306)	6.15	19·9	20·5	
6.4—6 6	25·58			292					
6.40—7.0		18·55 D	7.0	(324)		7.2	20·0	20·6	
6.58—7.0	25·46								
Averages............	25·79	19·00	Per hour 316·5 c.c.			Average.........		20·3	1·3 c.c.

solution by collecting 200 c.c. slowly (in two minutes) in the pipette W, and estimating its CO_2-content as described in Section I, f.

As in theory every particle of liquid takes about one hour to pass right through the apparatus, so any sample of effluent should strictly be compared with the sample of affluent taken an hour previously. When the composition of the affluent keeps fairly constant, as in this experiment, this point is not important, but if the affluent is altered in CO_2-content, either intentionally or by failure in the CO_2-generating tower, then it is important to bear this relation in mind.

For this particular experiment, only the *average* assimilation during six hours of the selected conditions is required, and the averages of the affluent and of the effluent liquids are taken, neglecting the first reading in either column of the table.

(b) *Data Required for an Exact Evaluation of the Assimilation.*—The magnitude of the following eight factors must be known numerically before a precise statement can be put forward about the assimilatory activity of the plant: (1) the weight of plant; (2) the intensity of illumination; (3) the temperature in the chamber; (4) the percentage of CO_2 in the circulating fluid; (5) the difference between the CO_2 in the affluent and effluent; (6) the rate of the current; (7) the CO_2 lost as gas-bubbles in the chamber; and (8) the magnitude of the plant's respiration.

We must say yet a little about the determination of each of these magnitudes in this particular experiment of June 7, 1907, before we pass to the calculation of the final result.

The unit of comparison for all cases and all plants in this method is the *quantity of leaf that will cover an arbitrary surface,* here 137 sq. cm., which is the area of the silver grid. In such a plant as Elodea the ideal unit, a unit area of the leaf-tissue itself, cannot be employed: one can only pack the shoots so closely (there is no objection to overlaying) that there are no gaps left where light is not being arrested, and then, however much leaf there is shaded and wasted as regards assimilation, there is yet the unit area of chlorophyllous cells exposed to the full illumination on the surface of the matted mass.

(1) The weight of plant required to cover the grid varies with its habit. It must in each case be ascertained in order to make the necessary allowance for the respiration, which varies with the total weight employed. As water-plants cannot be surface-dried satisfactorily, we have not taken the fresh weight, but have air-dried the material after each experiment, and have based the allowance for respiration on this dry weight.

(2) The standard light, at 13·4 cm., was used, having the arbitrary intensity 5·7 (see Section I, d).

(3) The temperatures of the bath and of the inside of the chamber were noted at intervals, as the entries in Table I show. The latter is always a little higher than the bath, due to the non-assimilatory radiation absorbed by the green leaf. The average temperature of the chamber, throughout the experiment, was 20°·3 C.

(4) The richness in CO_2 of the medium which bathes the leaves is of great importance. The number adopted must be the mean between the CO_2-content of the liquid entering the chamber and of that leaving it. The average of the last seven readings for the affluent gives 25·79 c.c. N/10 HCl as the content of 200 c.c. of the liquid entering the chamber. The corresponding number for the effluent is 19 c.c. So the mean content of the chamber equals 11·20 c.c. N/10 HCl per 100 c.c. of liquid = 0·0279 grm. CO_2 per cent. (1 c.c. standard acid being equivalent to 0·00249 grm. CO_2).

(5) The difference between the CO_2-content of the affluent and effluent gives in its uncorrected form the measure of the CO_2 which has disappeared from the chamber, chiefly, of course, by photo synthesis in the green tissues. Here it is 6·79 N/10 HCl = 0·01693 grm. CO_2 per 200 c.c. of solution, and this figure has to be calculated per hour (6), and corrected for bubbles (7) and respiration (8).

(6) The current rate is measured throughout the experiment by collecting all the effluent liquid in the measuring cylinders, F and G.

The outflowing liquid shifts from cylinder F to G every time the current is diverted from pipette D to E, and *vice versâ*, so that the volumes of effluent recorded in the table come alternately in F and G at rather irregular intervals of time. The actual volumes in the cylinders* are given in Table I and in brackets beneath each the rate per hour calculated from it. The average rate between 12.45 and 7 P.M. works out at 316·5 c.c. per hour. Therefore the weight of CO_2 lost per hour in the chamber is:—

$$0·01693 \times \frac{316·5}{200} = 0·02679 \text{ grm.}$$

(7) The amount of CO_2 escaping into the gas-bubbles formed must be ascertained and subtracted from the value in (6).

The gas evolved as bubbles was automatically collected over mercury in the receiver Z from 11.25 A.M. throughout the experiment as shown in the last column of the table. At 3.36 the amount collected was passed into the eudiometer for analysis. At laboratory temperature and pressure its total volume was 37·3 c.c., of which 4·8 c.c. proved to be CO_2; = 1·2 c.c. CO_2 per hour. At 6 P.M. the gas collected since 3.36 was again withdrawn and analysed. Its total volume was 27·4 c.c., containing 3·6 c.c. CO_2 = 1·5 c.c. CO_2 per hour. Therefore the average correction to be applied for the whole experiment was taken at 1·3 c.c. CO_2 per hour = 0·00239 grm. CO_2 (taking the weight of 1 c.c. CO_2 at laboratory temperature and pressure as 0·00184 grm.).

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* The contents of the cylinders are read off at frequent intervals to see if sufficient constancy is being maintained; if not, readjustment is made.

(8) During the experiment CO_2 is being formed in respiration both in the illuminated parts and in the shaded leaves in the background, *i.e.* by the whole *weight* of tissue present. Several separate experiments to determine the respiration were made at different temperatures, using the same procedure as in this experiment except that the bath was darkened. Some of these were made on Elodea before feeding up by assimilation, and others after several hours' preliminary assimilation, whereby the respiration is increased. The mean of the two values was taken as basis for a correction. The measurements of respiration were made at four different temperatures, and from these data a curve was constructed by which the respiration at any temperature within the range of the research could be derived.

After experiment the respiration plants were all dried and weighed air-dry and the respiration expressed per unit of dry weight. One gramme of dry Elodea has when fresh a respiration of 0·00125 grm. CO_2 per hour at 20°·3 C. Therefore the dry 1·955 grm. of Elodea used in this experiment involves a correction of 0·00244 grm. CO_2 per hour.

(c) *Final Calculation of Assimilation.*—We have now the data for calculating the corrected *Real Assimilation* proper to 137 sq. cm. surface of illuminated Elodea under the conditions of the experiment, viz., illumination = 5·7, average temperature of 20°·3 C. and CO_2-pressure in solution of 0·0279 grm. CO_2 per 100 c.c. (about 15 volumes of CO_2 per cent.).

CO_2 disappearing in chamber per hour	0·0268
Subtract CO_2 passing away in gas-bubbles	0·0024
Apparent assimilation	0·0244
Add CO_2 calculated for respiration at 20°·3 C. ...	0·0024
Real assimilation	0·0268

The corrections for bubbles and respiration are not large under these medium conditions, and by chance they happen to be equal and antagonistic, so that the total correction has no effect. Of course, this will not be so with solutions richer or poorer in CO_2.

This final number for the *Real Assimilation* of Elodea under this particular combination of environmental factors must be regarded as being very soundly established, as it is derived from the mean of six concordant hours of assimilation.

Each experiment of this kind is a whole day's work, but a considerable number have been put together in the years 1905, 1907, and 1908. The series dealing with the relation between assimilation and the CO_2-content of the liquid is fairly complete, and the results will be published in the next paper of this series.

(d) *Blank Experiments.*—We may conclude this section with a reference to one of the blank experiments made to test the accuracy and uniformity of the estimations. The course of this experiment is represented graphically in fig. 2:

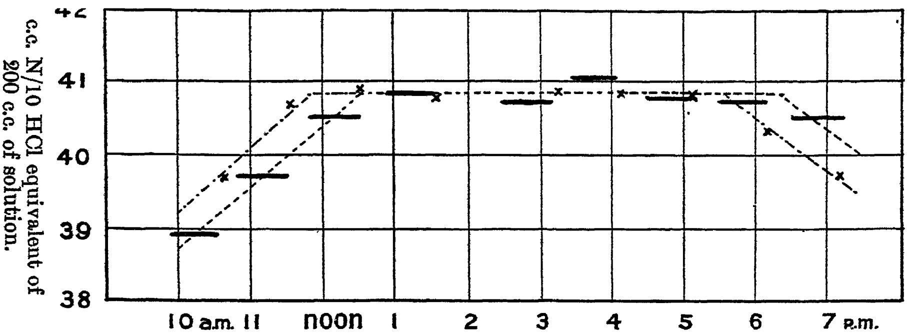

Fig 2

The procedure was exactly like that just described, but the chamber was set up empty, with no plant in it. The CO_2-solution prepared for the experiment was about quarter saturated, of such a strength that 200 c.c. of it equalled about 40·6 c.c. of N/10 HCl. The current was started at 9 o'clock (see fig. 2), and an estimation of the effluent was made every hour till 7 P.M. At the moment of the end of each collection of the effluent a sample of the affluent also was taken and analysed. Both sets of results are plotted in the diagram. Here the ordinates directly represent the equivalent in cubic centimetres of N/10 acid. The series of heavy horizontal lines represents the effluent collected in the pipettes D or E; as each contains 200 c.c., or the flow during 40 minutes, the lines drawn are of a corresponding time-length. The small crosses are the analyses of the affluent placed at the times when they were withdrawn in the pipette W. In this experiment the CO_2 solution was presumably not well mixed or the CO_2 generator at fault, for the strength of the solution rose steadily from 9 A.M. till noon. From noon to 5 P.M. uniformity was maintained, after which the strength of the solution fell off again. During the middle period the working is all that could be desired. The effluent and affluent analyses are practically identical all the time, which shows that there is no loss of CO_2 during the long journey of the water current, and that the general procedure is satisfactory.

Irregularities of current rate do not come into play in this form of experiment, and we reckon that the errors of a real experiment are bigger than this blank indicates, and may amount to perhaps 1 per cent., which is satisfactory enough.

The divergence of the effluent and affluent values at the two ends of the diagram as here represented is illusory, because it is merely due to the lag in the effluent on account of the hour or more it takes to flow through the chamber and apparatus. If the values of the affluent were all moved on an hour in the diagram they would coincide with those for the effluent right through the whole experiment. This blank experiment has been chosen because it illustrates the lag so well, which varies in different experiments from 40 to 80 minutes.

It must be borne in mind that it is not proposed to obtain critical values when the concentration of the CO_2 is rising or falling. Only when the series of analytical values of the affluent is nearly constant is the experiment considered a trustworthy one.

In conclusion we should mention that the cost of the important parts of the apparatus was provided by a Government grant from the Royal Society.

Experimental Researches on Vegetable Assimilation and Respiration. IX.—*On Assimilation in Submerged Water-Plants, and its Relation to the Concentration of Carbon Dioxide and other Factors.*

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INTRODUCTION.

The fourth paper of this series* contained an account of quantitative investigations on the magnitude of assimilation in the leaves of land-plants under different conditions of illumination and temperature.

The general conclusion reached was that the actual magnitude of assimilation in a leaf at any moment is determined by one or other of the main controlling conditions, light, temperature, or CO_2-supply, acting as a limiting factor.†

Since then it has been thought desirable to attempt a quantitative study of the assimilation in submerged water-plants on similar lines, and in particular to study the relation of assimilation to CO_2-supply, a subject which had been left on one side in the work on land-plants.

A preliminary difficulty was the absence of any satisfactory method of measuring, through a wide range of conditions, the assimilation of plants submerged in water. This difficulty has been removed by a new procedure which we have described in the previous paper of this series.‡ We may now proceed to consider some of the results obtained in this way.

The first section will deal with the factor of CO_2-supply, and the succeeding ones with the more general problem of how to disentangle the action of the different factors of the environment in any given case. In Section IV an attempt will be made to clear up and interpret the results of previous workers from our own point of view.

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* Blackman and Matthaei, 'Roy. Soc. Proc.,' B, 1905, vol. 76, p. 402.

† Blackman, "Optima and Limiting Factors," 'Annals of Botany,' 1905, vol. 19, p. 281.

‡ Blackman and Smith, "Assim. and Resp., VIII," 'Roy. Soc. Proc.,' B, 1911, p. 374.

Section I.—THE RELATION OF ASSIMILATION AND CO_2-SUPPLY.

In this section we give the results of our experiments on the effect of varying the factor of CO_2-content upon the assimilation of submerged water-plants. The greater part of this work has been done upon Elodea; in addition a series of experiments was made with the "water moss" *Fontinalis antipyretica* and a few isolated ones with Ceratophyllum and Potamogeton.

Our expectation in approaching this subject was that there would be only one *direct primary relation* between CO_2-supply and assimilation, namely that the assimilation should be directly proportional to the amount of CO_2 available. In some sets of conditions, however, this relation will not be realised, owing to the limit imposed upon functional activity by some other factor. Light, temperature, or the "time factor" may each act thus as a limiting factor. Working over a wide range of CO_2-content, it will therefore make all the difference to the results obtained what temperature and what illumination are selected for the series of experiments.

Our first series consists of 10 evaluations of the assimilation by *Fontinalis antipyretica* in different strengths of dissolved CO_2.

In Table I these experiments are arranged in order of increasing CO_2-content of the water flowing through the assimilation chamber. Each experiment proceeded exactly like the "typical experiment" described in "Assim. and Resp., VIII," Section II (a). For most of them medium temperatures and a medium illumination were employed. The number expressing the *real assimilation* in each experiment is the final value calculated in the way set out in "Assim. and Resp., VIII," Section II (c), and is fully corrected for CO_2 lost in gas-bubbles, and for CO_2 supplied by respiration.

The unit of tissue to which it applies is the standard area of 137 sq. cm. of illuminated leaf.*

In the lower part of the diagram—fig. 1—the values of the real assimilation in the series of experiments with Fontinalis are plotted against the CO_2-content of the surrounding water. This diagram presents several features of interest, and the broken line which represents the idealised relation of the successive values shows in a typical manner the anticipated effect of some limiting factor.

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* "Assim. and Resp., VIII," p. 384 *supra.*

Table I.

Plant.	CO_2-supply.		Light in arbitrary units.	Temp. of chamber, ° C.	Real assimilation, grm. CO_2 per hour, per standard area.	Date.
	Grms. CO_2 in 100 c.c. of water (mean).	Per cent. of saturation.				
Fontinalis	0·0080	4·91	8·1	22	0·0093	22.3.05
	0·0106	6·71	8·1	23	0·0133	17.3.05
	0·0124	7·85	5·7	23	0·0176	20.2.05
	0·0182	11·52	5·7	23	0·0237	15.2.05
	0·0236	14·93	5·7	23	0·0232	24.2.05
	0·0272	17·22	5·7	23	0·0218	21.2.05
	0·0326	20·63	5·7	23	0·0217	14.3.05
	0·0330	20·87	5·7	22	0·0230	20.3.05
	0·0338	21·39	5·7	23	0·0231	16.2.05
	0·0536	33·92	5·7	23	0·0223	14.3.05
Elodea	0·0028	1·58	8·1	19	0·0049	3.8.05
	0·0032	1·80	8·1	19	0·0083	4.8.05
	0·0054	3·05	5·7	19	0·0124	1.6.05
	0·0070	3·95	8·1	19	0·0205	1.6.05
	0·0078	4·40	5·7	19	0·0173	4.8.05
	0·0088	4·96	8·1	19	0·0276	1.6.05
	0·0090	5·08	5·7	19	0·0221	4.8.05
	0·0200	11·92	5·7	21	0·0226	21.8.08
	0·0202	12·04	5·7	21	0·0245	18.8.08
	0·0212	12·63	5·7	21	0·0252	20.8.08
	0·0279	16·11	5·7	20	0·0268	7.6.07
	0·0426	26·96	5·7	23	0·0216	26.5.05
	0·0490	37·50	5·7	30	0·0218	29.5.05
	0·0492	35·82	5·7	28	0·0249	30.5.05
Potamogeton ...	0·0080	4·63	5·7	20	0·0191	4.5.05
Ceratophyllum demersum ...	0·0014	0·77	8·0	19	0·0053	11.8.05
	0·0104	5·70	8·0	19	0·0321	5.8.05
	0·0120	6·58	8·8	19	0·0329	9.8.05

NOTES.—The values in Column 2 are the means between the amount of CO_2 in the water entering the chamber and the water leaving it. This is the most effective value of CO_2-content to take in relation to the assimilation. The numbers given in Column 3 are percentages calculated for the particular temperature of the individual experiment: they are therefore not strictly parallel with the absolute values in Column 2. (The data used were obtained from Landolt and Börnstein's Physico-Chemical Tables, p. 602, 100 c.c. of a saturated solution of CO_2 at 18° C. being taken to contain 0·1824 grm.) The light-intensities are calculated by the inverse squares of the distance of the front of the triple burner from the plant chamber, a distance of 13·0 cm. being taken arbitrarily as L.I. = 6 (*cf.* "Assim. and Resp., III," Sect. I (iii)).

In the weaker solutions of CO_2 the curve shows steadily increasing assimilation proportional to the increase of CO_2-supply. Here the light and temperature are in excess, but, at a certain point, sharply defined, increase of CO_2 is no longer followed by further increase of assimilation but the value of the assimilation remains at a fixed level of about 0·0227 (average of last seven values). This part of the curve is due undoubtedly to the limiting action of either the illumination or the temperature and presently experiments will be given which decide that it is due to the light.

Had a more intense light and higher temperature been fixed upon, then the ascending part of the curve would have been prolonged further and a fixed (but higher) level only attained with a greater concentration of CO_2. With less light the limiting value would have been arrived at sooner.

The second noticeable feature of this curve is the long range over which the constant limiting value is obtained. There is no sign of super-optimal depression of assimilation even with the strongest solution used.

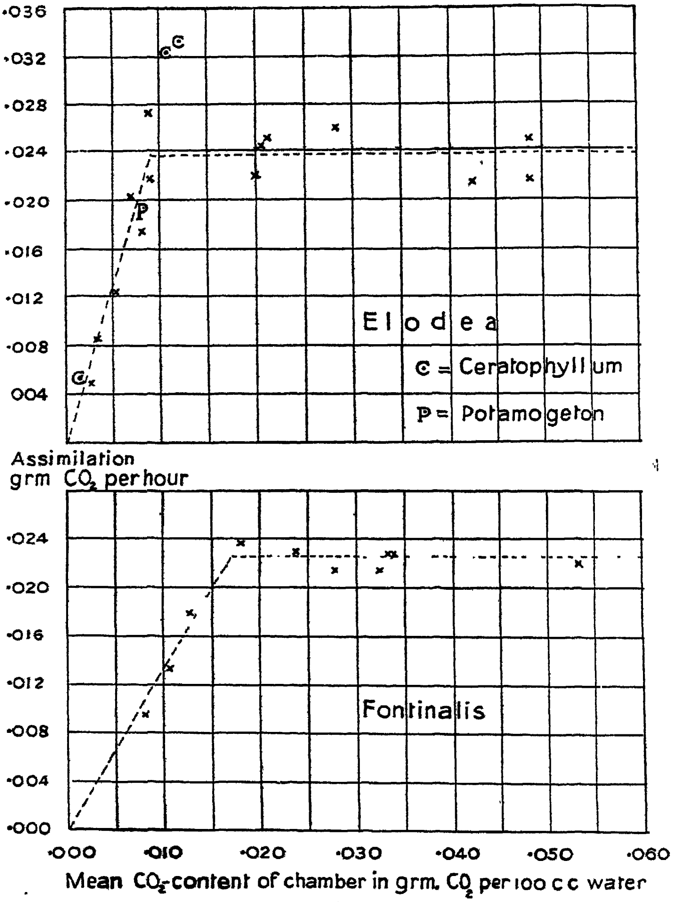

Fig. 1

In no previous experiments has the effect of such concentrated CO_2 been satisfactorily investigated and it affords strong support to our view that the whole conception of an optimum is out of place here, to find no signs of it even with an environment as rich in CO_2 as is an atmosphere containing 30 per cent. of this gas.

Of course with these aqueous solutions of CO_2, diffusion of this gas from the water in the chamber to the assimilating cells is much more sluggish than if the chamber were filled with a *gaseous* mixture containing 30 per cent. CO_2, so that in the latter the chloroplasts would probably be bathed in stronger CO_2 than in the former medium. No doubt we should then find a narcotic effect of the CO_2 on the protoplasm, though an aqueous medium containing 30 vols. per cent. dissolved CO_2 does not produce this effect.

Of the effect of subjecting the plants to still stronger solutions of CO_2 we shall speak briefly presently.

In the middle of Table I are found the results of a long series of experiments with Elodea and these data are represented by small crosses in the upper part of fig. 1. With Elodea the same form of curve is revealed as with Fontinalis; the limiting value is here slightly higher, 0·0237 (average of last eight values),* and until it is reached increase of CO_2 causes a proportional increase of assimilation.

With Elodea the assimilation values along the horizontal part of the curve are not so regular as with Fontinalis, but their agreement is perhaps as close as might be expected when it is noticed that the experiments range over three years and that identity for this value in successive experiments depends on repeating identically intense illumination of an identical area of leafy tissue.

The intensity of illumination varies with the square of the distance of the chamber from the light, and this had to be adjusted for each experiment but could not be measured directly, as the bath-window and cooling-screen intervened. An error of a few millimetres may have occurred and the light must have varied from changes in efficiency of the mantles. The uniformity of the area illuminated, 137 sq. cm., depended upon careful packing and distribution of the green shoots upon the silver grid; this was always covered as completely as possible, and the error from this cause is probably not great.

The difference of about 5 per cent. between the limiting values for the two plants has possibly no real significance at all and we may well hold that equal areas of different water-plants equally illuminated produce the same amount of assimilation, *provided* of course *that light is the limiting factor.* It may be recalled that this law was demonstrated in a previous paper† for a variety of types of land-plants and in that case also the agreement found experimentally was within 5 per cent.

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* The sixth value, 0·0276, is excluded from this average because a more intense light, = 8·1, was then employed, which would give too high a value should light 5·7 be limiting.

† Blackman and Matthaei, "Assim. and Resp., IV.," 'Roy. Soc. Proc.,' 1905, B, vol. 76, p. 444.

Considering only the low concentrations of CO_2, when CO_2 is certainly the limiting factor, there is, however, an interesting consistent difference between the assimilation-values for the two plants; one which holds for each concentration of CO_2. Fontinalis assimilates just about half the amount achieved by Elodea for any given concentration of CO_2, so it looks as if the obstacles to the diffusion of CO_2 up to the chloroplasts from outside were less in Elodea than in Fontinalis.

This difference seems to be a class distinction between the Bryophyte and the Phanerogam, for values obtained with plants of Potamogeton and Ceratophyllum are of the same order as those for Elodea, and quite different from those for Fontinalis. These experiments are given at the bottom of Table I, and the values for the real assimilation are incorporated in the top part of fig. 1. The four points marked P or C fall into perfect line with the crosses which represent the values for Elodea. The two highest values for Ceratophyllum, 0·0321 and 0·0329, are too high to be obtainable with light of 5·7 intensity, and it will be noted that the light was 8·0 and 8·8, due to its being placed closer to the plant. Thereupon the light ceases to be limiting, and the proportional relation to CO_2-supply is continued further than with the weaker light of 5·7. Ceratophyllum in 0·0014 grm. CO_2 solution, however, must be limited by the CO_2-supply, and so, although the light has the higher intensity, the assimilation value is down near that for Elodea in a similar solution.

The Bryophyte then is less efficient in utilising the CO_2 that surrounds it; possibly this may be the outcome of important structural differences, as submerged flowering plants have a well developed system of air-spaces containing an "internal atmosphere," while Fontinalis lacks this physiologico-anatomical characteristic.

This internal atmosphere certainly makes for greater efficiency in gaseous exchange generally, and the only doubt in accepting this theory of the cause of the difference between these plants arises from the fact that the leaves of Fontinalis are only one cell thick, and would therefore be expected to be highly efficient in assimilation.

A certain number of estimations of assimilation have been made with concentrated solutions of CO_2, exceeding those dealt with in the present paper and approaching saturation. With these concentrations there may be vigorous giving off of gas-bubbles; but, on analysis, the gas turns out to be mostly CO_2, and the corrected real assimilation is much depressed.

That, ultimately, depression of the assimilation sets in when the CO_2 is sufficiently increased is no evidence in favour of there being a *primary* *optimal* amount of CO_2 for assimilation; rather it is a sign of a general narcotic effect of the strong CO_2 upon protoplasm, and has no specific relation to assimilation. It is quite a general phenomenon, and it has been shown by previous workers that many vital processes are depressed by an atmosphere containing 20 to 25 per cent. of CO_2. Chapin* has given an account of previous work on CO_2-narcosis, and has himself shown that the growth of shoots and roots of higher plants is thus affected. He finds that the growth of bean-roots is retarded by 5 per cent. CO_2 and inhibited by 25 per cent.; with the hypocotyl of Sinapis, 15 per cent. retards and 25 per cent. suspends growth.

We conclude then that in the curve expressing, in any given light, the relation of assimilation to the whole range of CO_2-concentrations from zero to saturation, we may separate off the falling end-part of the curve as an effect of narcotic poisoning. This third and last phase thus contrasts with the first two phases, which are specific assimilation effects, the first rising in a straight line where the CO_2 is limiting and the assimilation proportional to it, and the second a horizontal line where the assimilation is limited by the light (or the temperature) and is independent of increase of the CO_2-supply.

Section II.—SUB-NORMAL ASSIMILATION OF UNHEALTHY PLANTS.

We must now point out that the results just dealt with are not the whole body of experiments done, but are selected results, and this selection has to be justified.

Many observers have noted that water-plants are very sensitive to unfavourable conditions of environment, and we have found marked depression of vigour and assimilation-power brought about by keeping plants in vessels of water in the laboratory. Even plants freshly collected from a natural habitat do not always show the same uniform vigorous activity: these sickly plants give low assimilation values and have been excluded from our tables and figures, which contain all the high normal results obtained under each experimental condition.

We may now give some examples of this sub-normal assimilation. For instance, on July 29th, 1905, as a test experiment, pieces of Elodea were gathered of quite dull and unhealthy appearance, lacking the bright green colour of the normal plants. The assimilation of this material was measured in the highest intensity of light (8·8) and in water containing 0·0416 grm. CO_2 per cent. In these conditions normal material would have given a value of about 0·034 grm. CO_2 assimilated per hour (consult fig. 1), but this unhealthy plant did not exceed 0·008 grm.

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* 'Flora,' 1902, Ergänzungsband, p. 348.

Such unhealthy plants would naturally be avoided in collecting, but it is not only those whose appearance obviously indicates loss of vigour that give figures below the normal for assimilation. Plants which have been kept in the laboratory shade-greenhouse in tap-water for a few days always show a falling-off in assimilation, though their outward appearance remains unchanged. For example, on May 26, 1905, a quantity of Elodea was collected, some experimented upon the same day, and the rest kept in a large basin of tap-water in the shade-greenhouse until June 6. The value obtained on May 26 was 0·0216 grm. CO_2 per hour in 5·7 intensity of light and CO_2-concentration of 0·0426 grm. per cent. (which figures in Table I as a normal value), while on June 6 under no less favourable conditions the value was only 0·0179, or about 17 per cent. less.

Again, Elodea gathered on June 8 which gave full active assimilation when measured on the same day gave on June 14 in 5·7 light and CO_2 strong enough not to be limiting (0·0318 grm. per cent.) only an assimilation value of 0·0167 grm. CO_2 per hour when at least 0·0240 would be normal. This falling off of nearly 30 per cent. was unaccompanied by any microscopic sign of change, and was brought on by five days' stay in a basin in the laboratory and 24 hours in the dark in the actual assimilation-chamber* before the assimilation value was measured.

It is reasonable to conclude that among a large number of random gatherings of healthy-looking Elodea from natural habitats some will be depressed below their normal vigour, and therefore it seems more instructive to eliminate from the final comparison of assimilation values, those which fall considerably below the general level under similar conditions.

Section III.—THE INTERACTION OF LIMITING FACTORS.

When the two series of experiments described in the first section were carried out in 1905 we were not certain whether it was the light or the temperature employed that limited the assimilation of both Elodea and Fontinalis to approximately the same value in all the higher concentrations of CO_2. The temperatures lay between 19° and 23° C., except in two cases, and the light intensity was usually 5·7. Inspection of the table would incline us to the view that it was the light that was limiting because, in the two cases where temperatures of 28° and 30° C. were employed with Elodea, no higher assimilation was noted than with much lower temperature at the same light intensity, 5·7.

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* This long enclosure in the chamber may have been specially deleterious; it was never repeated.

There were also other small indications in the same direction.

When this work was taken up again in 1907 and 1908 definite experiments were made to determine what limit was actually set to assimilation by the light and the temperature employed respectively in the long series of earlier experiments. Five such experiments, all made with Elodea, may now be described briefly, and these will serve to exemplify the general principle that is employed for experimentally determining which of the possible factors is actually limiting the function in any specific case. This principle may be formulated as follows:—*When several factors are possibly controlling a function, a small increase or decrease of the factor that is limiting, and of that factor only, will bring about an alteration of the magnitude of the functional activity.*

Each of our experiments accordingly will involve the change of some one factor in the middle of the experiment to test whether the assimilation responds to this change or not.

Experiment A: CO_2-Supply the Limiting Factor.—In this case the light intensity was 8 and the temperature 19° C. throughout the experiment. The CO_2-supply was at first 0·0032 grm. CO_2 per 100 c.c. solution, and at 3 P.M. it was suddenly increased. The diagram in fig. 2 shows the course of the assimilation. As in all experiments in this section the abscissæ are time and the ordinates grammes CO_2; two separate curves are given, one for the assimilation (in grammes CO_2 assimilated per hour), and the other for the CO_2-supply in grammes CO_2 dissolved per 100 c.c. of the solution.*

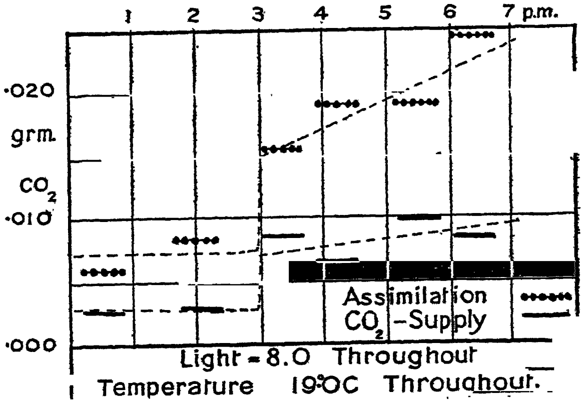

Fig. 2 Exp. A.

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* It is, perhaps, slightly unfortunate that the weight of CO_2 assimilated is greater than the number given for the CO_2-supply. This, of course, is only a question of units, as the plant has in each hour *three* hundred cubic centimetres of the solution to draw from.

The mean of the two assimilation estimations, each representing 40 minutes, taken before 3 o'clock is 0·0073 grm. CO_2 per hour. With so low a CO_2-supply we expect this factor to be the limiting one, and at 3 P.M. the supply bottle of CO_2-solution was opened and filled up with a stronger solution. Owing to excessive generation of CO_2 in the tower, and perhaps also to imperfect mixing in the supply bottle, the final strength of the new affluent was only slowly attained, continuing to increase until 7 P.M. The behaviour of the assimilation after the change shows clearly that, in this particular combination of factors, the CO_2-supply actually was limiting, for the assimilation increases suddenly at 3 P.M., and goes on rising continuously till 7 P.M., about in proportion to the altering the CO_2-supply.

Experiment B: CO_2-supply the Limiting Factor.—In this case the temperature was 19° C. throughout, and until 5 P.M. the light was 6. At 5 P.M. the light was increased to 8·3. It was intended to maintain the CO_2-supply at 0·0090 grm. per 100 c.c. throughout, but, as the diagram of fig. 3 shows, its concentration declined in the last half of the experiment.

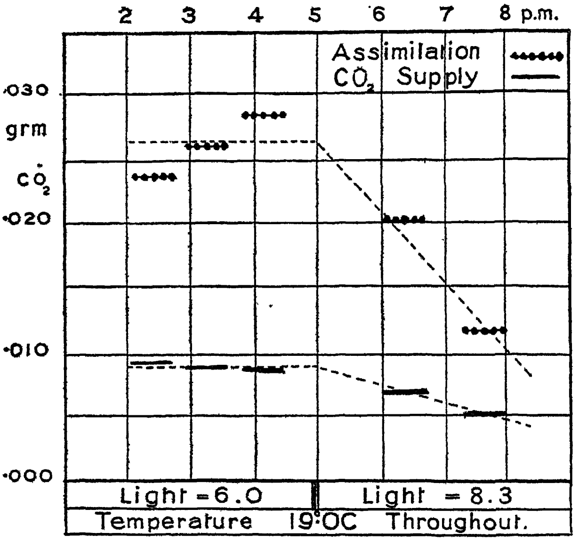

Fig. 3 Exp. B.

The assimilation values for the first half average 0·0265, which might possibly be limited by either the light or the CO_2 (see fig. 1). On increasing the light it becomes clear that the latter is true, because not only is there no increase of assimilation, but there is a decrease due to the fall that has set in with regard to the CO_2-concentration.

Experiment C: Light the Limiting Factor.—An experiment was carried out at a temperature of 29° C. and a CO_2-supply of 0·0490 grm. per 100 c.c., both of these factors being very high. In the first half the light intensity was only 4·2, being increased to 5·7 at 6 P.M. (fig. 4). The assimilation from 4 to 6 P.M. averaged only 0·0183, much lower than either CO_2-supply or temperature would account for, and so it was expected that the light was limiting. This was proved in the second half of the experiment, because on increasing the light the assimilation rose, giving an average of 0·0220 between 7 and 9 P.M., which Table I shows us is a possible limiting value for the light of 5·7.

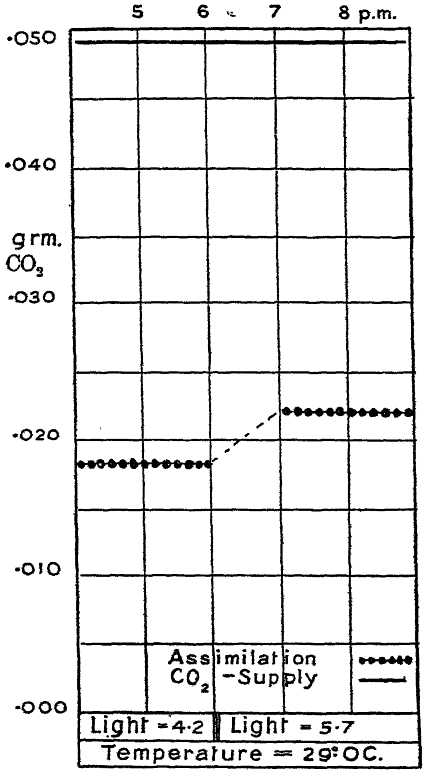

Fig. 4 Exp. C.

We have attempted to prove directly that the average value of 0·0237 in the series on Table I is limited by light by starting an experiment in some of the conditions there shown and subsequently *increasing* the light still further, in the expectation of getting still greater assimilation.

There is, however, a difficulty about doing this, because rates of assimilation much higher than 0·0240 are not very long maintained before a decline sets in. This is a general phenomenon in assimilation which has elsewhere* been attributed to the "time factor," which term we may continue to use as a non-committal name for the collective action of certain internal depressant factors still obscure.

An increase of illumination in the middle of an experiment superposed on a natural decline of assimilation might partly or wholly neutralise this decline and yet not lead to an actually increased assimilation. We have, indeed, shown that the progressive decline in time is less marked when the light intensity is increased than when it is constant, but the experiments are not significant enough to be given in detail here.

Experiment D: Temperature the Limiting Factor.—The light was of 5·7 intensity throughout and the CO_2-supply 0·0230 to 0·0215. The experiment started at a temperature of 7° C., which was maintained by the careful addition of ice to the bath at short intervals; at 4.12 P.M. the bath was quickly warmed up and kept at about 21° C. from 5 to 7 P.M. (see curve of temperature of chamber in fig. 5). At the low temperature the assimilation remained at 0·0115 hour after hour, not half the value that this combination of light and CO_2 permitted in 1905. It is clear then that this value marks

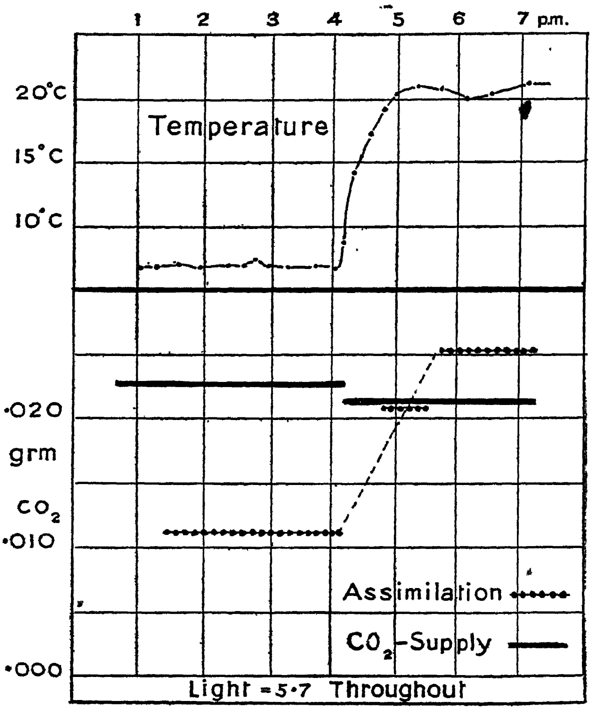

Fig. 5 Exp. D.

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* "Optima and Limiting Factors," p. 282.

the limit set by the temperature of 7° C., and on raising the temperature the assimilation rose to 0·0252; the conditions of 1905 being thus reproduced, the assimilation rose to a value which falls into line with those of that year in Table I.

Experiment E: Temperature the Limiting Factor.—A second experiment similar to D was then carried out in order to get the limiting value for some other temperature. In this case the light was 5·7 and the CO_2-supply 0·0212, and at first the chamber was kept at a temperature of about 13° C. Fig. 6 shows that the assimilation kept at 0·0177 for three hours under these conditions. At 4.30 P.M. the temperature was raised to 21° C., and then between 6 and 8 P.M. the assimilation increased to 0·0226, a low, but possible, value for the light as limiting factor, as Table I shows.

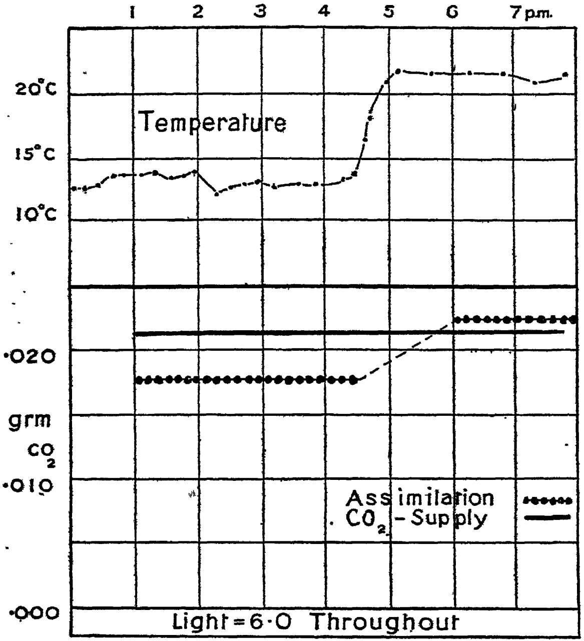

Fig. 6 Exp. E.

The bearing of these experiments upon the problem of the nature of the limiting factor in the two long series in 1905 is clear.

By Experiment D we have established 0·0115 as the "specific temperature maximum" for the temperature of 7° C., and by E the value of 0·0177 for the temperature of 13° C. Assuming that assimilation in water-plants increases logarithmically with temperature, which can hardly be doubted on the evidence available for land-plants, we can from these two data calculate the coefficient of increase for 10° C. The coefficient arrived at is $K_{10} = 2{\cdot}05$.*

We are now in a position to calculate the *primary* curve of assimilation and temperature in Elodea (see fig. 7, middle curve), and this will hold *actually* for medium temperatures. From inspection of this we see that the lowest temperature used in 1905, namely, 19° C., should allow an assimilation of at least 0·0267. The average limiting value obtained was, however, less, being 0·0237, so that though this is not much below the temperature limit it is just enough so for us to consider it certainly proved that the light of 5·7 intensity and not the temperature (19° to 23° C.) was the limiting factor in the 1905 series of experiments.

The Inter-relation of these Limiting Factors.—We will conclude this section by showing how the knowledge of the interaction of limiting factors on assimilation here set out will give us the power of predicting the value of the assimilation that will take place in any selected combination of the three external factors.

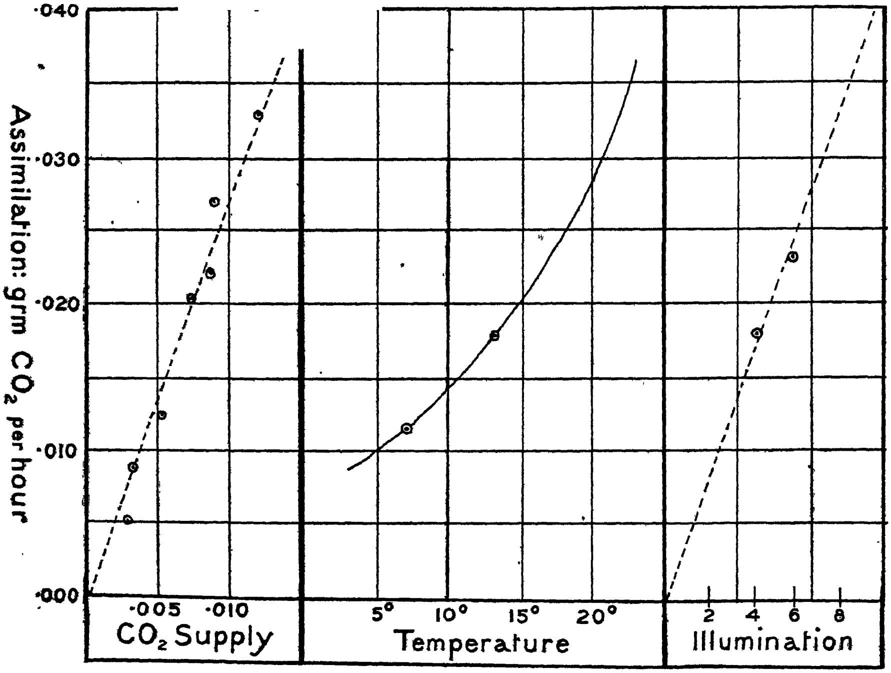

Fig. 7.—Schema of inter-relation of factors and assimilation in Elodea.

Fig. 7 shows for Elodea the relation between different magnitudes of assimilation and each of the three factors, CO_2-supply, temperature, and illumination separately.

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* This is nearly identical with the coefficient $K_{10} = 2{\cdot}1$ arrived at for Cherry Laurel, "Optima and Limiting Factors," p. 283.

The two latter curves are based on the data set out in this section, the particular points established being marked with circles; the curve for CO_2-supply is taken from fig. 1. We can by inspection tell exactly what minimum of CO_2, temperature, and light will be required for any one of the values of assimilation on the ordinates by following the horizontal line right across the diagram and noting where it intersects the three curves respectively.

Reciprocally, given any hypothetical combination of different magnitudes of the three factors, we have only to note in the diagram the values of assimilation corresponding to each of them, and we can foretell that the value of assimilation actually attained in that combination will be *the lowest of the three values.*

Section IV.—CRITICAL ANALYSIS OF THE RESULTS OF PREVIOUS WORKERS.

Instead of prefacing the present paper by the usual summary of previous work, we have thought fit to expound our own point of view first, and we now proceed to some critical account of the work of other investigators. This order is adopted because we differ from them in the interpretation to be put upon the facts of assimilation rather than in the nature of the facts themselves. We may confine ourselves to the works of Reinke,* 1883; Treboux,† 1903; and Pantanelli,‡ 1903.

Reinke counted the bubbles given off by Elodea in spring water with a little added CO_2, when it was exposed to a series of lights of different intensity§ in rapid succession. After the plant had been about one minute in a given illumination the number of bubbles liberated in two successive quarter minutes was counted, and then the intensity of the light was altered and a new reading started. Increasing the light from 1/24 to 1/2 sunlight caused generally a proportional increase in the rate of bubbling, but further increase of light, even up to 64/1 sunlight, gave no further increase of bubbling, but a maintenance of the same uniform rate as at 1/2 sunlight.

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* J. Reinke, "Untersuchungen ü. d. Einwirkung des Lichtes auf die Sauerstoffausscheidung der Pflanzen," 'Bot. Zeit.,' 1883, p. 697.

† O. Treboux, "Einige stoffliche Einflüsse auf die Kohlensäure-Assimilation bei submersen Pflanzen," 'Flora,' 1903, p. 53.

‡ E. Pantanelli, "Abhängigkeit der Sauerstoffausscheidung belichteter Pflanzen von äusseren Bedingungen," 'Jahrb. f. wiss. Bot.,' 1903, vol. 39.

§ Reinke placed his plant, within a dark room, in the path of a beam of sunlight concentrated by a large lens of long focus. By adjusting the distance of the plant beyond the focus of the lens he obtained positions corresponding to a series of definite illuminations, *i.e.*, 1/36, 1/25, 1/16, 1/9, 1/4, 1/1, 4/1, 9/1, 16/1, 25/1, 36/1, 49/1, and 64/1, these being fractions of the direct sunlight falling at the moment on the lens from a heliostat. Pantanelli used also the same method of obtaining definite intensities of light.

To us this is a perfect example of the action of CO_2-supply as a limiting factor; the temperatures were between 20° C. and 28° C., and therefore too high to be limiting with this small (though indeed unknown) CO_2-supply.

Reinke naturally, at that time, regarded the long level part of the curve as being in direct relation to the intensity of light and as being a *prolonged optimal effect.*

It should be mentioned here that Pfeffer, in 1897,* suggested that possibly the photo synthetic work of the Elodea in Reinke's experiments was limited by the inadequate CO_2-supply, and that if adequate CO_2 were supplied, an optimal relation between light and assimilation might become evident. This first suggestion of a specific factor limiting the activity of assimilation was not developed further.

Treboux, in Pfeffer's laboratory, worked chiefly at the effect of drugs, acids, and salts upon the bubbling of Elodea, using the light of one or two Auer burners at a temperature of about 16° C. in a dark room; he has, however, in addition a section upon the influence of the quantity of dissolved CO_2. His procedure was to change, every fifteen minutes or so, the water in which the Elodea was bubbling for a water containing twice the previous quantity of CO_2. All the CO_2-concentrations were low, beginning at 0·1 vol. CO_2 per cent., and doubling up to 12·8 vols. CO_2 per cent. His series of bubble-countings show that at first the rate doubled with the doubled CO_2-supply, but that abruptly the increase ceased, and further concentrations of CO_2 gave continually only the same value.

On carrying out a similar experiment with a doubled light throughout (Experiment XVIII), the progressive doubling of assimilation was continued one stage longer, and then a maximal value was reached—at about 3 vols. CO_2 per cent.—and maintained unaltered.

Treboux concludes (1) that assimilation is proportional to CO_2-supply, and (2) that with weak lights the CO_2-optimum shifts proportionally with the light-intensity. Treboux's observations are to us also a clear case of the CO_2-supply at first being a limiting factor to the assimilation, and then the feeble light in its turn acting as one. These results are just the converse of Reinke's, and both are according to expectation.

The work of Pantanelli, also finished in Pfeffer's laboratory, must be considered in more detail. It is the latest and fullest work which deals with the effect of the factors of light-intensity and CO_2-supply upon assimilation in water-plants. Pantanelli's ultimate object was the investigation of the effects of intense light upon chloroplasts and their power of assimilation. He is interested to see whether the decline of assimilation in "ultra-optimal" light is correlated with bleaching of chlorophyll, aggregation of plastids, and inhibition of protoplasmic circulation; and he rightly attaches great importance to the functional activity of the colourless protoplasm of the plastid. Too readily, however, he shelves complex phenomena as due to "irritability," and interprets his effects generally in terms of fatigue of the plastid by the strong stimuli of intense light. He would define the optimum as the maximal intensity of function which can be carried on for an appreciable time without fatigue.

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* 'Physiology,' vol. 1, sect. 59.

We propose to show how, in our opinion, the data which Pantanelli presents in illustration of his principles can be more clearly and satisfactorily interpreted from the point of view of interaction of limiting factors. Let us take first the curve which he puts forward for the relation of the rate of bubbling to different intensities of light. On Plate 4, Elodea, *a*, Pantanelli gives a graphic diagram which represents the bubbling in a series of lights ranging from 1/36 sunlight to 64/1 sunlight, being the mean of all the experiments carried out in less than 15 vols. CO_2 per 100 c.c. water.

The enclosed part of the diagram in our fig. 8 represents Pantanelli's curve in slightly modified form (*cf.* p. 408); the abscissæ are intensities of light, and the ordinates the times in seconds for the liberation of 10 bubbles.

Pantanelli's interpretation of his results being based upon the conception of optima, he naturally finds it difficult to apply such interpretation precisely over a wide range of conditions. He has no thought of abandoning this point of view as untenable, and he is therefore driven to hold that the optimum of one factor may be shifted considerably by alterations in the magnitude of the other factors, so that the position of the CO_2-optimum becomes purely relative to the magnitude of the light-intensity, and so on.* This can only be regarded as a transitional point of view and cannot be even a temporary halting place.

The lights he investigated ranged from 1/36 sunlight to 64/1 sunlight (see footnote, p. 408) and the CO_2-supply from 1 to 50 vols. per 100 c.c. of water. As regards the factor of temperature we have unfortunately inadequate information, because Pantanelli's research was carried out before

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* He says: "The light optimum for Elodea, etc., is about 1/4 sunlight in spring water, and shifts towards stronger light with increase of the CO_2-content, and towards weaker light with decrease of CO_2"—p. 224. "With 1/4 light CO_2-optimum is about 10 vols. CO_2 per cent.; with 1/1 light, 15 vols. CO_2 per cent.; and with 4/1 light, about 20 vols. CO_2 per cent."—p. 195.

Miss Matthaei* had caused the great significance of this factor to be generally recognised. Pantanelli is satisfied with stating that his experiments were all done between 22° C. and 30° C., within which range temperature was then believed to be without any determining effect on assimilation. In the course of each single experiment, however, the temperature of the water is stated to have varied very little.

In Pantanelli's experiments the small sprig of Elodea, 1 cm. long, was contained in a large cubical glass vessel of CO_2-laden water, and this was moved along through a series of increasing or decreasing light intensities, staying exactly 10 minutes in each position.

The first point for comment is that the rate of bubbling did not remain constant minute after minute in each position, but nearly always fell off steadily all the time. This effect is slight in weak lights but strongly marked in intense lights.

This falling off in time may be partly due to external factors† and partly to internal causes which may be aggregated as the "time factor."

An effective cause of decline is to be found in the protoplasmic disturbance produced by the intense radiation of lights 16 to 64 times the intensity of direct sunlight. The excess light absorbed by the chlorophyll will cause considerable local heating of the plastids, which will depress their activity generally, as may also its direct photo chemical action. In accordance with this view it is only with these higher intensities of light that the falling off is so marked. Further, the effect is cumulative in Pantanelli's procedure because Elodea is not exposed to light 64/1 without having first stayed ten minutes in each of the lights 9/1, 16/1, 25/1, 36/1, and 49/1. Thus the assimilation value for each intensity is depressed by the sum of the injurious effects of all the previous members of the series, and cannot be brought forward as the value of assimilation really corresponding to that intensity of light.‡

With such a series of changing values, there arises at once the question as to which value is to be taken as best representing numerically the assimilation proper to that set of conditions. Pantanelli arbitrarily assumes that

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* Matthaei, "Assim. and Resp., III," 'Phil. Trans.,' 1904, B.

† One may wonder whether the stagnation of the water and slow diffusion of CO_2 had anything to do with these falling values. Prolonged experiments in one vessel of water certainly need artificial stirring. In Pantanelli's apparatus the water could be made to flow through the vessel, but it is not clear that it was his practice to have it so. F. Darwin and D. M. Pertz have shown how striking is the effect of stirring ("On the Effect of Water-currents on the Assimilation of Aquatic Plants," 'Camb. Phil. Soc. Proc.,' 1895, vol. 9, p. 76).

‡ No attempt is made to get information on this matter by determining, for comparison, the rate of bubbling when Elodea is exposed straight away to 64/1 without passing slowly up the whole series of lights.

ten minutes is the proper time to allow for the chloroplast to readjust itself to a new set of conditions. For this there does not seem to be any real experimental justification in his work, and in his experiment on p. 182, where the plant continued 15 minutes in each light, the values continue to fall steadily for the whole 15 minutes.

To us it seems that the better value to take would be the earliest value that is not subject to any special suspicion. The initial values recorded in each light-intensity are in all cases also the highest values, when the series of lights is an ascending one, and it is the initial values of bubbling that we have taken in the presentation of Pantanelli's curve given in the inset diagram in our fig. 8.

The first part of this curve is represented by a double track, because the experiments always began with the Elodea in light 1/1, and then proceeded by ten-minute stages down to light 1/36, after which the vessel, with its sprig of plant, is returned by the same stages to 1/1, and so on to 64/1. In further dealing with this curve, we propose to simplify this by taking the mean of the up and down series from 1/1 to 1/36 and back (see note to fig. 8). In such weak lights there is no injurious effect, and the plant returns practically to the same value for 1/1 as it started with.

For lights 1/1, 4/1, and 9/1 the same initial value (within ±2 per per cent.) is obtained in all three cases, afterwards the initial values begin to get lower and lower, not due to a primary optimal point having been passed, but to cumulative depression from prolonged exposure to intense radiation.

If this is a sound point of view, one would expect that the high initial values would be kept up further along the series if the plant stayed a less time at each stage. Pantanelli's own results show that this is so, for on p. 181 there is a small diagram of a set of experiments in which the plant stayed only three minutes in each position. Here the initial values up to 36/1 are practically the same as at 1/1, and those at 49/1 and 64/1 are only a little less.

Even the curve of initial values, as modified in the inset to fig. 8, does not give the right aspect of the relation investigated, and this is due to the distorted scale of abscissæ and ordinates adopted.

It will be noted that from 4/1 to 64/1 the abscissæ are proportional to the light-intensity, but for intensities below 1/1 down to 1/36 a logarithmic scale is adopted, which would put the zero of light at an infinite distance. Further distortion is produced by the fact that the ordinates do not represent *intensity of assimilation* (*i.e.* rate of bubbling), but the time taken by 10 bubbles to form, which is the inverse of the

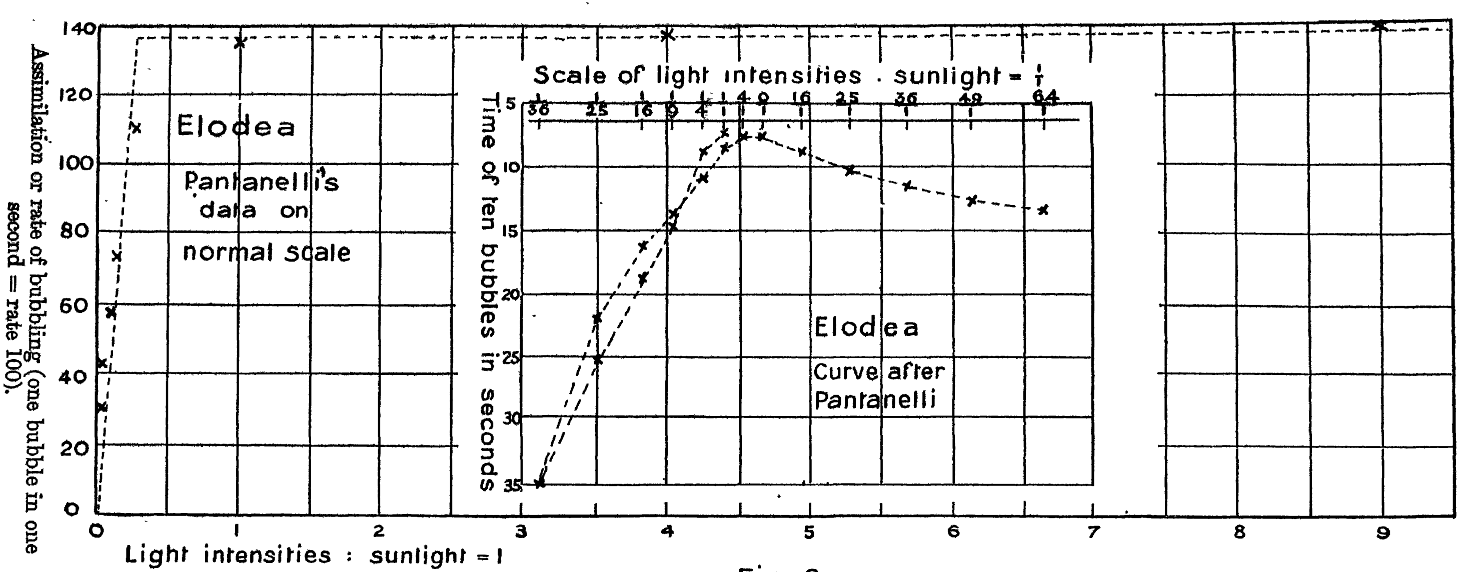

Fig. 8

The enclosed diagram gives, on a much reduced scale, Pantanelli's curve (Plate IV, Elodea, *a*), simplified by taking the initial value only for each intensity and omitting the subsequent ones. His exact data are appended to show the derivation of our outer curve from them.

	Light Intensities.												
	1/36	1/25	1/16	1/9	1/4	1/1	4/1	9/1	16/1	25/1	36/1	49/1	64/1
	Time of ten bubbles in secs., Initial Values. (Pantanelli.)												
Descending series	35	2[illegible]	19·2	15	8·5	7·1							
Ascending series	35	21	15	12·3	9·6	7·7	7·3	7·2	8·2	10	11	12	13
Mean values............	35	23·5	17·1	13·7	9·0	7·4	7·3	7·2	8·2	10	11	12	13
	Rate of bubbling: Inverses of above: 10 bubbles in 10″ = Rate 100. (B. and S.)												
	29	42	58	73	111	135	137	139	122	100	91	83	77

rate, and here again the zero of bubbling would be at an infinite distance. Such methods of presenting facts are obscurantic rather than graphic, and we have been compelled to construct the larger outer curve of fig. 8 to give the corrected relation of the initial assimilation-values in the different intensities of light.

Inspection of this curve at once shows that we are dealing with a limiting factor relation such as we have found in our own work. From intensity 1/36 to 1/4 the assimilation increases in direct proportion with the increase of light and then a limit is reached. Possibly the limit is set by the CO_2-supply, which did not exceed 15 vols. CO_2 per 100 c.c. of water (0·03 grm. CO_2 per cent.). On the other hand, it may have been due to the temperature, but we cannot go into this matter as there is no clue to the temperature at which this particular batch of experiments was carried out.

In spite of this uncertainty, it is impossible not to conclude from the form of the curve that a limiting factor is at work. Consequently, no higher assimilation is ever reached, though the light is increased twenty-seven fold, up to 9/1.

Pantanelli's curve of the relation of bubbling-rate to light-intensity is therefore only really a curve of this nature as regards its first ascending part—1/36 to 1/3 light. The next part, 1/3 to 9/1, has nothing to do with light, but represents the assimilation corresponding to the limited CO_2-supply or temperature. Finally, we regard the third falling part (lights 16/1 to 64/1, shown only in the inset diagram to fig. 8) as not a specific assimilation effect at all, but as exhibiting the cumulative effect of depression of protoplasmic activity.

As a corollary to his investigation of the light-optimum, Pantanelli also made experiments on assimilation, with constant illumination and varying CO_2-supply, to obtain information upon the location of the CO_2-optimum.

This, he says, he found much less easy to investigate, as simple bubble-counting is not satisfactory in strong CO_2-solutions. He therefore only gives details of three single serial experiments in which, starting with a constant light-position, say 1/4 sunlight, the bubbling rate of a shoot of Elodea was observed in a succession of cylinders of water with different amounts of dissolved CO_2, the plant staying 10 minutes in each medium.

Pantanelli gives (p. 194) the initial and the final timings for the liberation of 10 bubbles, during the 10 minutes stay in each concentration of CO_2, for the most satisfactory of his experiments, but there is no curve. We have constructed one (fig. 9) from his data, selecting, in each strength of CO_2, the more rapid of his two rates, as sometimes the final rate is recorded as quicker than the initial.*

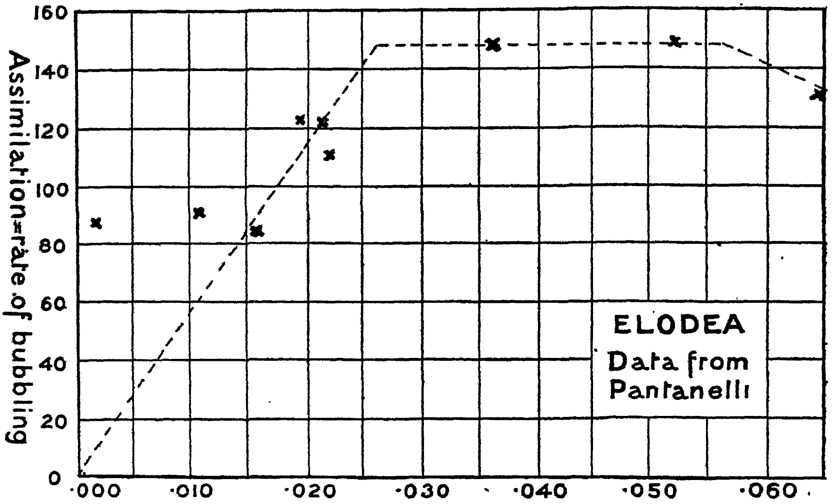

FIG. 9.

The data are not very regular, but it cannot be doubted that the diagram is of the same nature as our own fig. 1. Up to about 0·020 grm. CO_2 per 100 c.c. the assimilation increases proportionally with the CO_2-supply; then a limit is set by the 1/4 light-intensity, and the assimilation remains constant till about 0·06 grm. CO_2. After this the narcotic effect of strong CO_2 seems to begin, as bubbling in 0·065 grm. CO_2 falls distinctly lower (this is just outside the range of our experiments in Section I). Further than this, as Pantanelli points out, the investigation cannot be pushed by bubble-counting alone as the physical liberation of CO_2 from the strong solution is so great.

In this series, with intensity of light = 1/4, Pantanelli places the optimum at 0·02 grm. CO_2 (10 vols. CO_2 per cent.)†; in a similar series, with 1/1 light, at 0·03 grm. CO_2, and in the third series, with 4/1 light, at 0·04 grm. CO_2.

We trust that our detailed consideration of Pantanelli's work justifies itself by proving that there have been no facts brought in support of the conception of optima in connection with assimilation which are not more harmoniously interpreted on the theory of the interaction of limiting factors.

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* The bubble rate in "CO_2-free" water (= 0·002 grm. CO_2 per 100 c.c.) is impossibly high, but it is entered in the diagram; the rate in the strongest CO_2 (No. X) is obviously physical and erratic, and is omitted from the diagram.

† On p. 193 Pantanelli has an interesting footnote in which he treats with incredulity Treboux's location of the CO_2-optimum in his particular experiments at so low a value as about 3 vols. CO_2 per cent. This is obviously explained by the fact that Treboux used such a weak light (see p. 404), that the plant was inevitably prevented from utilising much CO_2.

Section V.—CONCLUSIONS.

1. The experiments on assimilation in water-plants dealt with in this paper are carried out by a new method which takes account of the alteration of the gases in solution as well as of the gases liberated as bubbles.

2. The aim of this study is to demonstrate the *nature* of the relation between assimilation and the chief environmental factors; (1) CO_2-supply, (2) light-intensity, and (3) temperature. The relation is such that *the magnitude of this function in every combination of these factors is determined by one or other of them acting as a limiting factor.*

3. The identification of the particular limiting factor in any definite case is carried out by applying experimentally the following general principle. *When the magnitude of a function is limited by one of a set of possible factors, increase of that factor, and of that one alone, will be found to bring about an increase of the magnitude of the function.*

4. Two long series of experiments with Elodea and with Fontinalis have been carried out in which the light and temperature remained constant while the CO_2-supply varied through a wide range (from 0·0025 to 0·0540 grm. CO_2 per 100 c.c. of water).

In both series the same phenomena is observed; at first the assimilation increases steadily in proportion to the increase of the CO_2-supply and then abruptly this increase comes to a stop, at a value of about 0·023 grm. CO_2 assimilated per hour. This is shown to be the limit set by the particular intensity of light adopted and, without more light, no further increase of CO_2 will bring the assimilation above this level.

5. The form of curve obtained is a typical "compound limiting factor curve"; there is no sign of an optimum or of ultra-optimal depression of assimilation even when water one-third saturated with CO_2 is employed.

The first phase only of this curve exhibits the significant *primary relation* between CO_2-supply and assimilation, and it is clear that here the magnitude of assimilation varies in direct proportion to the CO_2-supply.

6. Incidentally it comes out that the Bryophyte, Fontinalis, is consistently less efficient in the intake of CO_2 than the submerged Phanerogam, Elodea. This is possibly correlated with the absence of any "internal atmosphere" in the former.

7. From the data obtained in this research there is constructed a diagram,

fig. 7, by which it is possible to foretell what value of assimilation in Elodea will be attained in any combination of medium magnitudes of the three factors of the environment. In this diagram, against the different values of assimilation as ordinates, are ranged three separate curves showing the degrees of CO_2-supply, temperature and illumination, which are respectively essential for the attainment of each value of assimilation. For any hypothetical combination of the factors, it follows, by the principle of limiting factors, that if the three functional values corresponding potentially to these be ascertained from the diagram, then the actual magnitude of assimilation attained with that combination of factors will always be *the smallest of the three potential values.*

8. The experiments in this paper deal with such moderate intensities of assimilation as may be fairly well maintained for several successive hours. With more intense assimilation the values soon fall off by the action of internal factors grouped at present as the *time-factor.* Experiments in which this additional factor has to be reckoned with will be considered in a later paper.

9. Section IV contains a critical account of the work of previous investigators who interpreted their results on the assumption that there was *a primary optimum* in the relation between assimilation and each external factor. The substantial work of Pantanelli (1903) led him to the conclusion that the position of the optimum for any one factor shifts with the magnitude of the other concurrent factors. This can only be regarded as a transitional point of view and from this we have advanced to the conclusion that *the whole conception of optima in this connection is inapplicable* and breaks down completely on careful analysis. It is shown in detail that all the experiments of previous workers are more harmoniously interpreted from the point of view of interacting limiting factors than by the conception of optima.

Certain Physical and Physiological Properties of Stovaine and its Homologues.

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(From the Physiological Laboratory of the University of London.)

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I. INTRODUCTION.

The amino-alcohols of the general type $(OH)CCH_3RCH_2N(CH_3)_2$ were originally obtained by Fourneau* by the reaction between the secondary or tertiary amines and the corresponding chlorhydrins $(OH)CCH_3RCH_2Cl$ (R representing any hydrocarbon grouping). Their salts do not crystallise well, but if the hydrogen of the hydroxyl grouping be replaced by benzoyl, the derived salts, especially the hydrochlorides, can be obtained in a pure crystalline condition. Such salts produce local anæsthesia, the ethyl derivative, $OBzC.CH_3.C_2H_5CH_2N(CH_3)_2HCl$, or stovaine, being especially used in surgical practice. In a former communication by Veley and Waller,† the activities of stovaine, its methyl homologue, and cocaine were compared as regards their effect on the contractility of isolated muscle; it was shown that the toxic effects of the three drugs were all equal within the limits of experimental error, but the variation of effect by altering the concentration was rather less than that to be expected.

A few months later, Gros,‡ using a different method of excitation, namely, tetanic, obtained similar results as regards the equal activity of cocaine and stovaine, though the concentrations in the two cases were widely different, namely, *n*/500 to *n*/1000 (Veley and Waller) and *n*/12·5 (Gros).

M. Fourneau recently sent to the former of us fine crystalline samples of stovaine and its homologues for the purpose of investigation; we desire to express our appreciation of his kindness and international courtesy.

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* 'Comptes Rendus,' 1904, vol. 138, p. 766.

† 'Roy. Soc. Proc.,' B, 1910, vol. 82, p. 147.

‡ 'Archiv f. Exp. Pathol. u. Pharmakol.,' Leipzig, 1910, vol. 62, p. 380.

The substances sent were—

(i) Methyl stovaine,* $OBzC(CH_3)_2CH_2N(CH_3)_2HCl$;

(ii) Ethyl stovaine, or stovaine, $OBzC.CH_3.C_2H_5.CH_2N(CH_3)_2HCl$;

(iii) Amyl stovaine, $OBzC.CH_3.C_5H_{11}.CH_2N(CH_3)_2HCl$;

(iv) Phenyl stovaine, $OBzC.CH_3.C_6H_5.CH_2N(CH_3)_2HCl$;

(v) Benzyl stovaine, $OBzC.CH_3.C_7H_7.CH_2N(CH_3)_2HCl$; and

(vi) The propyl ester of a dimethyl-amino-oxybenzoyl-isobutyric acid, $OBzC.CH_3.COOC_3H_7.CH_2N(CH_3)_2HCl$, which differs from stovaine in containing the grouping $COOC_3H_7$ in the place of the ethyl (C_2H_5) group. As regards the corresponding methyl ester, Fourneau† wrote:—"Il contient tous les groupements de la cocaine" (namely a tertiary amino, an oxybenzoyl, OBz, and a methylated carboxyl, $COOCH_3$, grouping) "c'est d'ailleurs un anæsthésique puissant, mais l'accumulation des groupes acides autour de la fonction aminée détruit tellement la basicité de celle-ci que la molécule est très acide au tournesol." In other words, the salt may be regarded as intermediate, as regards its constitution, between stovaine on the one hand and cocaine hydrochloride on the other, though, as regards its properties, differing from both of them in giving an acid reaction; this point will be more fully discussed in the sequel.

II. PHYSICAL PROPERTIES.

The physical properties of the several compounds investigated are—(i) the densities of the salts themselves; (ii) the affinity value of Fourneau's salt, and, as to the physiological properties, their effects on (i) the contractility of isolated muscle, (ii) blood pressure, and (iii) respiration. The comparative effect of these drugs on nerve is dealt with as a separate subject in a further communication.

Densities of the Salts.—These were determined by displacement of toluene, using a specific gravity bottle of 10 c.c. capacity, rather more than 1 gramme of each salt being used; the necessary correction for air displacement was made, and each experiment performed in duplicate; a bottle of like capacity and nearly identical weight was used as a tare. It is not, of course, pretended that such determinations are of the order of accuracy which can be obtained in the case of liquids with a U form of pycnometer, but the curious absence of data for the densities of salts of organic compounds was an inducement to arrive at some values. The following results were obtained in terms of water at 15°:—

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* For the sake of brevity, the words "homologue of stovaine" will be omitted in the case of the compounds (i), (iii), (iv), and (v); for the same purpose, compound (vi) will be designated in the text as Fourneau's salt.

† 'Journ. de Pharmacie et de Chimie,' June 1, 1908; also 'Bull. Soc. Chim.,' 1908, [4], vol. 3, p. 114; and *ibid.*, 1909, vol. 5, p. 239.

Table I.

Salt.	S.G. 15/15.	Molecular weight.
Methyl stovaine	$1 \cdot 213_6$	257·5
Ethyl "	$1 \cdot 207_6$	271·5
Amyl "	$1 \cdot 106_0$	313·5
Phenyl "	$1 \cdot 191_2$	335·5
Benzyl "	$1 \cdot 178_0$	349·5

(The variation in the results obtained in the duplicate experiments was in the fourth decimal place.)

It is evident from the above results that the densities, both for the paraffinoid and benzenoid derivatives, decrease with increase of molecular weight, but this decrease does not vary uniformly with each CH_2 group added to the molecule; the value of the amyl derivative is lower than that to be expected, but four determinations were made for the salt in the case of any error.

Affinity Value of the Base of Fourneau's Salt.—On account of the writer's results as to the acidity of the salt towards indicators the hydrolysis and affinity values were determined by the methyl-orange method* devised by the former of us. The hydrolysis found was 1 per cent. at dilution $V = 4 \times 10^3$; hence the affinity value of the base K_b can be calculated by Arrhenius' dilution formula and Kohlrausch's data for ionisation of water as

$$K_b = 0 \cdot 99 \times 4 . 10^3 \times 4 \cdot 9 . 10^{-15} / (0 \cdot 01)^2 = 7 \cdot 9 . 10^{-8}.$$

The above value is comparable with that of glycine ethyl ester hydrochloride $= 9 \cdot 7 . 10^{-8}$, and also for such comparison the affinity values (as determined by the borax method) of cocaine, stovaine, and methyl stovaine, whose hydrochlorides show no hydrolysis, are given below:—†

Base.	$K_b . 10^{-7}$.
Cocaine	4
Stovaine (base)	1·5
Methyl stovaine (base).........	3·2

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* 'Trans. Chem. Soc.,' 1908, vol. 93, pp. 652, 2114, 2122.

† 'Trans. Chem. Soc.,' 1909, vol. 95, pp. 763—766.

Fourneau's view that the accumulation of acidic groupings around the amino-function destroys the basic character of the latter is confirmed by quantitative measurements.

III. PHYSIOLOGICAL PROPERTIES.

A. *Contractility of Isolated Muscle.*

On dissolving in the usual manner in physiological tap-water saline solution, a turbidity resulted in the case of the salts of the higher molecular weight; this was due to the precipitation of the base by the alkali of the water, as previous investigations have shown that, though these compounds are not hydrolysed by water, yet a trace of the order of 1 per 10,000 of free alkali will cause separation of the base. But if such solutions are allowed to stand for some days, the turbidity slowly and almost completely disappears except in the case of benzyl stovaine.

The records obtained for all the compounds were similar in type, the only features worthy of note being (1) that the end or abolition point was often not very well defined, and (2) that the variation caused by alteration of concentration was less marked than for most other drugs.

The times required for abolition are collected together in the following table; in Column I the names of the compounds are given, in II, III and IV the concentrations in normality, and the same in percentage reckoned as base, and the times in minutes required for abolition. The temperature, unless otherwise stated, was 18 ± 0·5°.

Table II.

I. Compounds.	II. *n*/1000.		III. *n*/500.		IV. *n*/300.	
	Percentage.	Time.	Percentage.	Time.	Percentage.	Time.
		mins.		mins.		mins.
Methyl stovaine	0·022	23·5	0·044	18	0·077	—
Stovaine	0·024	23·5	0·048	17·5*	0·081	6·5 (at 21°)
Amyl stovaine	0·028	—	0·056	24	0·098	9·0 (at 21°)
Phenyl stovaine	0·030	—	0·060	24	0·100	11·0
Benzyl stovaine	0·031	—	0·062	—	0·104	12·0 (at 20°)
Fourneau's salt	0·029	30	0·058	24	0·097	11·5

* Three determinations made at different times gave identical values.

The general conclusion to be drawn from these results is that the substitution of the ethyl grouping in stovaine by the amyl, phenyl, benzyl, and

($COOC_3H_7$) groupings produces a decrease of rate of action while that of methyl is less marked, the differences observed being within the limit of experimental error. As regards the amyl, phenyl, and benzyl derivatives, it is possible that the differences observed may be due to the precipitation of the bases as pointed out above.

B. *Effect on Blood-pressure and on Respiration.*

Though it was not possible to carry out, *in vivo*, a complete investigation into the physiological action of these bodies, it nevertheless seemed of interest to employ some of the material at our disposal for the purpose of comparing their respective effects, with those of cocaine, on the circulation and respiration of the mammal.

The number of observations is not large enough to warrant close quantitative estimation of their individual toxicities, but suffices to enable comparison of the type and (to some extent) of the degree of their effects.

In all cases the animals employed were cats, under complete anæsthesia, and the drugs were administered by injection into the saphenous vein.

Cocaine.

As is well known, cocaine in sufficiently small doses is a cardiovascular and respiratory stimulant. On cats with intact central nervous system, blood-pressure was raised by doses of 0·05—0·7 mgrm. per kilo. body weight, lowered by larger doses; whilst on a completely pithed cat injections produced no fall in doses up to 1·9 mgrm. per kilo. On the respiratory movements, the depressant action of the drug has appeared with smaller doses than on the blood-pressure.

Stovaine and Methyl Stovaine.

These drugs have not, with us, raised blood-pressure or stimulated respiration in any dose. Small doses, when effective, led to fall of blood-pressure of varying amount and duration, together with a momentary pause in respiration, which was followed by a rhythm of greater frequency and smaller amplitude than that before injection. A single (first) dose of stovaine amounting to 5 mgrm. per kilo. has proved fatal, but provided that the initial doses were small, single injections amounting to three times as much did not cause death, and a total dose of 40 mgrm. per kilo. can be tolerated. With methyl stovaine an initial (single) dose of 8·4 mgrm. per kilo. did not produce any obviously dangerous effect. As on muscle* and on nerve,* these drugs have also on the circulation and on the respiration similar and approximately equal effects, methyl stovaine being, if anything, slightly the less active of the two.

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* Veley and Waller, *loc. cit.*

Amyl Stovaine.

In comparison with the two preceding drugs, small doses of amyl stovaine have produced (molecule for molecule) a greater fall of blood-pressure, but distinctly less interference with respiration.

Initial doses of 5·8 and 9·3 mgrm. per kilo. were well tolerated, and 41 mgrm. per kilo. has failed to kill. The effects are neither in type nor in degree, those of an acutely toxic drug. We have found amyl stovaine to be a powerful local anæsthetic.*

Phenyl Stovaine and Benzyl Stovaine.

Only one observation was made in each case. With phenyl stovaine a brief rise of blood-pressure preceded the invariable fall, and both drugs produced some dyspnœa. With benzyl stovaine Traube-Hering and Cheyne-Stokes rhythm was noticed in the blood-pressure and respiration respectively. Both drugs are powerful local anæsthetics, but offer no advantage over amyl stovaine.

Fourneau's New Salt.

As already pointed out, this body is (in chemical structure) intermediate between cocaine and stovaine. In its effects, on injection, it also resembles both these drugs, the latter more closely than the former. Small doses may (or may not) produce a trifling initial rise of blood-pressure, but any such rise is small in comparison with the slight fall that invariably follows it. This fall is less than that caused by equivalent doses of the preceding drugs. On respiration, its effect closely resembles that of stovaine and of methyl stovaine, but the momentary pause is followed by closer return to the normal rhythm. Its effects on blood-pressure and on respiration are therefore less marked than those of cocaine, stovaine, and the homologues of the latter. As elsewhere† stated, it is a powerful local anæsthetic.

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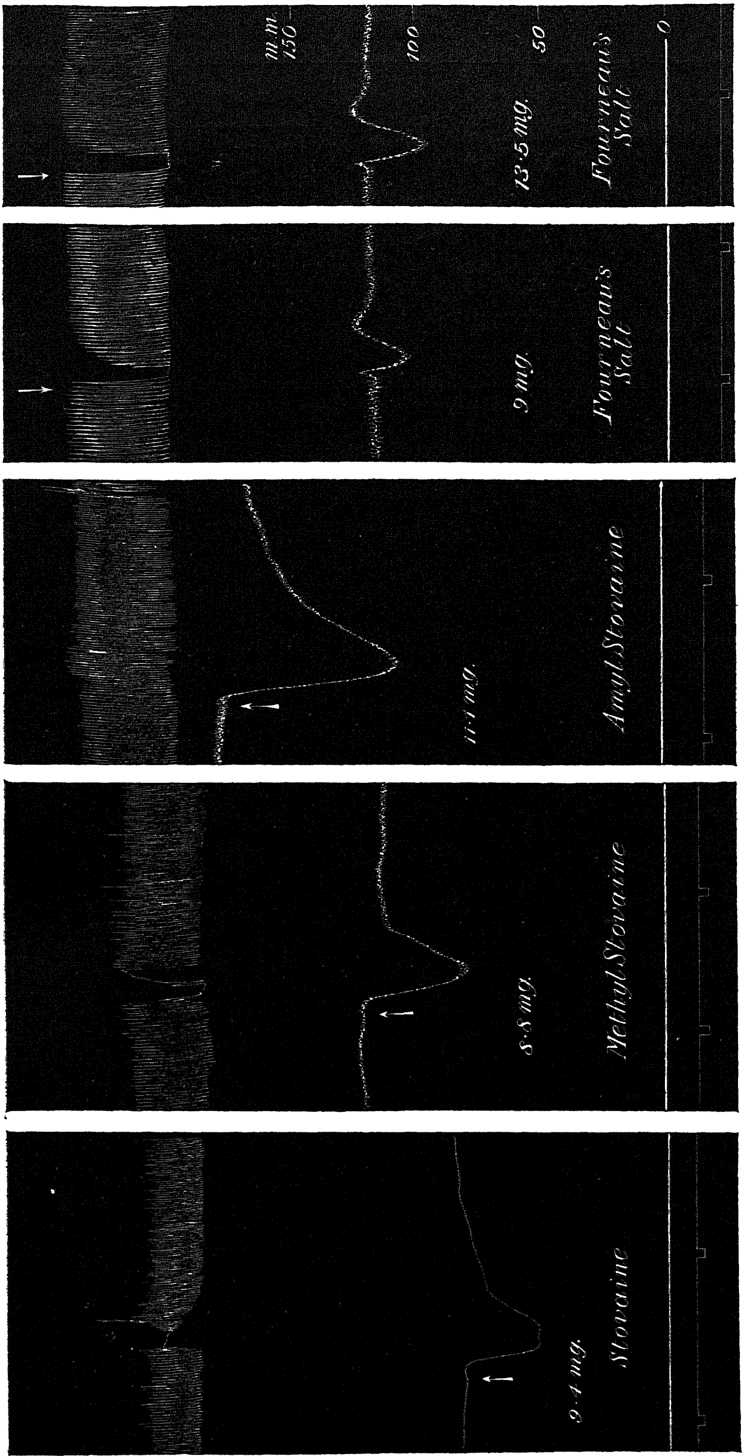
* *Vide infra*, p. 426.

† *Infra*, p. 427.



Effect of intravenous injections on respiration and on arterial pressure. Each injection is signalled by an arrow. Time tracing shows minutes. For other details consult Table III.

Table III.—*Cf.* Fig.

Drug.	Weight of animal.	Mean arterial pressure.		Ratio of fall to original pressure.
		Before injection.	After injection.	
	kgrm.	mm. Hg.	mm. Hg.	Per cent.
Stovaine (9·4 mgrm.)......................	3·7	80	52	35
Methyl stovaine (8·8 mgrm.)	3·3	122	82	33
Amyl stovaine (11·1 mgrm.)............	2·8	176	108	39
Fourneau's new salt (9·0 mgrm.)......	3·0	116	106	9
" (13·5 mgrm.) ...	3·0	118	96	19

These results are shown in the records—Fig. 1.

The above doses of stovaine, methyl stovaine, and amyl stovaine are equimolecular to one another and to 11·6 mgrm. of Fourneau's salt. The effect of amyl stovaine is perhaps exaggerated by the smallness of the animal.

In short, on the central nervous system and on the circulation, Fourneau's salt is less active, and methyl stovaine is, at most, no more active, than stovaine. On the central nervous system, amyl stovaine is less active; on the circulation, its depression is somewhat greater in degree, though more gradual in onset.

These three bodies will be further investigated, on more nearly clinical lines, for the purpose of ascertaining whether their anæsthetic properties may fittingly be employed in medical and surgical practice.

The Effect of some Local Anæsthetics on Nerve.

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(From the Physiological Laboratory of the University of London.)

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I. Introduction.

The employment of stovaine, in clinical practice, to produce local anæsthesia by direct application of the drug to nerve (lumbar anæsthesia) suggested comparison of this substance with its homologues,* as also with cocaine, on a similar plan. Afferent nerves, however, were not chosen for the purpose, as their use would have involved the ambiguities inseparable from quantitative examination of reflex action. Hence motor nerves, with their simpler index of activity (muscular contraction), were taken in preference.

In principle, the plan adopted was identical with that used by Grünhagen† and others in studying the effect of gases and vapours on the conductivity of nerve, viz., applying the agent under examination to the nerve between the stimulating electrodes and the muscle. Wedenski,‡ on the same lines, has investigated the effect of painting solutions of cocaine (and of other drugs) on a motor nerve trunk, and more recently Dixon,§ and Tait and Gunn‖ have respectively tested cocaine and yohimbine by analogous methods.

This method is advantageous in that it entails no disturbance of the stimulated portion of the nerve, and enables study of the modes of onset and of subsidence of the anæsthetic block under constant excitation. It was found possible, with as many as six excitations per minute, to observe these two processes, on the same nerve-muscle preparation, within times sufficiently short to exclude fatigue.

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* The physical properties of these bodies and their effect on skeletal muscle, on respiration, and on circulation are dealt with in the preceding paper, p. 413.

† Grünhagen, 'Arch. f. d. ges. Physiol.,' 1876, vol. 6, p. 180.

‡ Wedenski, *ibid.*, 1900, vol. 82, p. 134; and 1903, vol. 100, p. 1.

§ Dixon, 'J. Physiol.,' Cambridge, 1905, vol. 32, p. 87.

‖ Tait and Gunn, 'Quart. Journ. Exp. Physiol., London,' 1908, vol. 1, p. 191.

The local anæsthetics which we have compared in this way are cocaine, stovaine, the methyl-, amyl-, phenyl-, and benzyl-homologues of the latter, and a new salt prepared by Fourneau, viz., the propyl ester of a dimethylamino-oxybenzoyl-isobutyric acid, which as elsewhere* pointed out, is intermediate in chemical structure between cocaine hydrochloride on the one hand, and stovaine on the other. In addition to their bearing on the comparative effects of individual drugs, our results throw light on the response of nerve to direct, and on that of muscle to indirect, electrical stimulation.

II. METHOD OF EXPERIMENT.

Most of our observations were made on the isolated nerve-sartorius, with a light myograph, in a moist chamber. The stimuli were maximal break induction shocks, applied to the nerve at intervals of 10 seconds, just below its divergence from the main sciatic trunk.

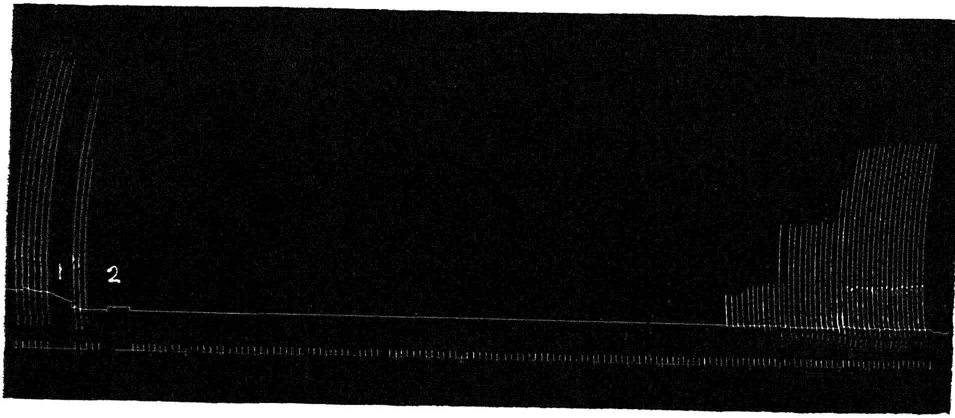

FIG. 1.—Blockage of Conductivity (local anæsthesia) in nerve by Fourneau's new salt N/100 = 0·29 per cent. solution.

. . . 1 Nerve in normal saline—preliminary test of preparation.
1 . . . 2 Nerve in anæsthetic solution—onset of blockage.
2 . . . . Nerve in normal saline—restoration of conductivity.

The lower line signals minutes (down strokes) and stimuli (up strokes).

Between the electrodes and the muscle, approximately 2 millimetres from the latter, about 1 centimetre of the nerve was immersed in a bath containing successively:—

A. Normal saline solution (0·6 per cent. NaCl in tap water).
B. A solution of one of the drugs in normal saline.
C. Normal saline again.

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* Veley and Symes, *supra*, p. 414.

During these periods the muscle twitches were recorded on a drum moving about 1 cm. per minute, whilst a double electro-magnetic signal (Locke's model), writing below the muscle tracing, served to record time and stimuli. The inductorium was either (A) a Cambridge model which, with signal in primary circuit and a current of 0·6 ampère from a single accumulator, was used with the secondary coil at 175 mm., or (B) a model of Berne type which, under the above conditions, carried 0·4 ampère and was used with the secondary 400 mm. from its zero. In both cases the stimulus was well in excess of that necessary to evoke maximal response from a good nerve-muscle, and any preparation that failed to respond maximally was rejected.

III. CONDITIONS WHICH INFLUENCE THE ANÆSTHETIC BLOCK.

It was to be expected that the rate of diffusion of the drugs into the nerve would influence the rate of onset of the block. This was substantiated by our preliminary tests, in which we found that an N/100 solution of cocaine hydrochloride required 40 minutes to produce on the sciatic-gastrocnemius an effect attained, with the same solution, on the nerve-sartorius, in about one-twentieth of the time. So great a difference shows that, even with the same nerve from different frogs, variations in the rate of blocking may be expected from minor variations in the position of the motor fibres relatively to the surface exposed to the drug.

Individual nerves differ in quality; *e.g.* a fresh preparation from a fresh frog is blocked more rapidly than a preparation either stale or made from a frog that has been kept in stock for some time. As would be anticipated, increase in concentration of the drug solution, or repetition of the drug bath soon after recovery from a block, leads to more rapid abolition of conductivity. With dilute solutions of the drugs, decrease in temperature, when sufficiently great, delays the onset of the block, especially in the case of cocaine, but between 16° and 20° C. the effect of temperature is often masked by other factors.

For a given position of the secondary coil, a block which is complete to maximal break shocks is almost invariably complete to tetanic stimulation. When not so, the response to the latter is seldom tetanus, but the brief clonus or apparently simple twitch noticed by Wedenski, and later by Tait and Gunn (in the case of yohimbine), and is rarely obtainable more than once.

Moreover, when a block is once complete, the stimulus may be considerably increased without provoking further response. With inductorium A, a block with the secondary coil at 175 mm. was invariably a block when the secondary was pushed up to zero, both for single shocks and for tetanic stimulation. With inductorium B, a block with the secondary coil at 400 mm. was always complete at 300 mm. and at 200 mm., though not always at 100 mm., and when not so, was noticeably less effective against tetanisation than against single stimuli. In such cases, prolongation of the drug bath was necessary to abolish response with the secondary coil at zero.

On substituting normal saline for the anæsthetic solution, when the block is complete, response remains for a time in abeyance, but sooner or later returns.

The rate of recovery of the nerve is affected by the factors that influence the onset of the block. Increase in concentration of the drug, early repetition of the drug bath, and pronounced fall in temperature, all lead to delayed recovery, whilst a fresh nerve recovers (as it fails) more rapidly than a stale one.

Furthermore, recovery is delayed by prolonging the duration of the drug bath after response has ceased.

IV. Effects of the Individual Drugs.

Cocaine Hydrochloride.

In comparison with stovaine and its homologues, cocaine hydrochloride blocks somewhat less rapidly and allows much more rapid recovery. Moreover, it is the most conspicuously affected by dilution, N/500 (0·07 per cent.) solutions acting with extreme slowness (Table I).

Table I.*—Cocaine Hydrochloride.

Concentration.	Temperature.	Time required to block maximal stimulus.	Duration of block.
	° C.	mins.	mins.
N/100 (0·34 per cent.).........	18	2	2
" "	10	6·5	15
N/300	18	2	<1
"	20	1	<1
"	20	1	3·5
N/500	18·5	22	<1
"	18·5	53	<1

Gros† has shown that addition of sodium hydrate to a solution of cocaine hydrochloride accelerates its effect on the excitability of nerve, and attributes this to liberation of some of the cocaine base. In agreement with his results, we find that such addition also quickens the effect of this drug on nerve conductivity, and further that it prolongs the duration of the resulting block. But it is obvious that Gros' solution[*] is too complex to warrant his explanation of this acceleration.

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* In this and the following tables second and subsequent observations on the same nerves are not included.

† Gros, 'Archiv f. Exp. Pathol. u. Pharmakol.,' 1910, vol. 63, p. 80.

In our hands solutions of cocaine base have acted much less rapidly than equimolecular solutions of its hydrochloride.

We have found this both with Merck's cocaine (base) and with the base which we prepared from our solutions of (Merck's) cocaine hydrochloride, by precipitating with ammonia, washing free from chloride, and drying the solid. We have also found the hydrochloride, re-formed from this precipitated base, to possess its original activity. These results are shown in Table II.

Table II.—Comparison of Cocaine Base and its Hydrochloride, N/300 in each case.

Base (Merck)	No block in 10 mins.			
Salt („)	Complete block in		2·5	mins.
Base (precipitated)	„	„	12·5	„
Salt (Merck)	„	„	1·5	„
Salt (re-formed)	„	„	1·2	„

Stovaine.[†]

With stovaine, the block ensues more rapidly and lasts longer than with equimolecular solutions of cocaine hydrochloride. Further, more dilute solutions are effective, and temperature has less influence than with the latter drug. Gros (*loc. cit.*) also noticed that stovaine abolished excitability of nerve the more rapidly, and that this abolition was accelerated by traces of alkali, a fact which he explains as in the case of cocaine. We have obtained effects analogous to this, but find the acceleration less marked than with the cocaine salt.

As the base of stovaine is an oil, that does not lend itself readily to purification, we have not compared its effects with those of the hydrochloride.

On muscle and on the electrical response of nerve, Veley and Waller[‡] found cocaine hydrochloride and stovaine to act with practically equal rapidity.

---

[*] N/100 cocaine hydrochloride, 10 c.c.; N/100 sodium hydrate, 8 c.c.; 0·7 per cent. sodium chloride, 2 c.c.

[†] We are indebted to M. Fourneau for our samples of stovaine and its homologues.

[‡] Veley and Waller, 'Roy. Soc. Proc.,' B, 1910, vol. 82, p. 147.

Table III.—Stovaine.

Concentration.	Temperature.	Time required to block maximal stimulus.	Duration of block.
	°C.	mins.	mins.
N/100 (0·27 per cent.)	18	1	4
" "	10	2	4
N/500	17	<1	7
"	17	4	3
N/600	19·3	9	<1
N/1000	19·3	17	<1
"	28	3	<1
"	18	4	<1

Methyl Stovaine.

Methyl stovaine closely resembles stovaine in rate of action,* but its effect subsides more rapidly when the nerve is replaced in saline. Its affinity value has been found by one of us† to be somewhat greater than that of stovaine. It may therefore offer some advantage over the latter, since its base is less readily precipitated by faintly alkaline solutions such as cerebro-spinal fluid or lymph.

Table IV.—Methyl Stovaine.

Concentration.	Temperature.	Time required to block maximal stimulus.	Duration of block.
	°C.	mins.	mins.
N/100 (0·26 per cent.)	17·8	1	>20
N/300	20	11	8
"	17·2	3·5	1
N/500	20	1·5	<1
"	17·2	6	<1

Amyl Stovaine.

Close comparison between this and the preceding drugs is difficult owing to gradual precipitation of its base by tap water.‡ Solutions in tap-water saline act more slowly than equimolecular solutions of stovaine, or of methyl stovaine, but the resulting block is of greater duration. In distilled water saline, it both acts and is washed out rather more rapidly, though less so than the other drugs mentioned above. It, therefore, offers the advantage of more sustained effect against the possible disadvantage of more ready precipitation of its base.

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* *Cf.* Veley and Waller, *loc. cit.*

† Veley, 'Trans. Chem. Soc.,' 1909, vol. 95, p. 758.

‡ Solutions of amyl stovaine are more readily decomposed by boiling in soft glass vessels than are those of stovaine.

Table V.—Amyl Stovaine.

Concentration.	Temperature.	Time required to block maximal stimulus.	Duration of block.
	° C.	mins.	
N/100 (0·31 per cent.)	18	2·5	<2 hrs.
N/300	19	3	<24 mins.
N/500	17·4	8·5	6 „

Phenyl Stovaine and Benzyl Stovaine.

The bases of these bodies are even more readily precipitated by alkalis than that of amyl stovaine. It was, therefore, impossible to examine them in tap water saline. In distilled water saline their effect resembles that of amyl stovaine in the relative slowness of its onset and subsidence. They appear to offer no advantage over amyl stovaine.

Table VI.

Concentration.	Temperature.	Time required to block maximal stimulus.	Duration of block.
	Phenyl Stovaine.		
	° C.	mins.	mins.
N/300 (0·1 per cent.)	19	1	>27
N/500	19	<1	5
„	16·5	8·5	1
„	18·5	7·8	<1
	Benzyl Stovaine.		
N/300 (0·12 per cent.).........	16·5	2	27
N/500	18·5	4	5·5
„	15·6	10	8·5

Fourneau's New Salt.

This body is one of the most active of the series. In rapidity of onset, and in duration, its effect is comparable with that of stovaine. As stated above, p. 418, it is relatively inactive on the central nervous system and on the circulation. It should therefore prove an admirable local anæsthetic. According to Fourneau (*vide* footnote, p. 414) its methyl homologue also possesses marked local anæsthetic power.

Table VII.—Fourneau's Salt.

Concentration.	Temperature.	Time required to block maximal stimulus.	Duration of block.
	° C.	mins.	mins.
N/100 (0·29 per cent.)	15·6	<1·0	16·0
N/200	15·6	1·2	6·5
N/300	16·0	2·0	10·0
N/500	21·0	7·0	5·0
„	21·0	2·5	>16·0
„	17·0	2·0	2·0

V. GENERAL CONSIDERATIONS.

The records of failure of the muscular response to stimulation of its motor nerve (by maximal break induction shocks of constant intensity), conditioned by failure of conductivity in the latter, are of the same type with all the drugs that we have tested.*

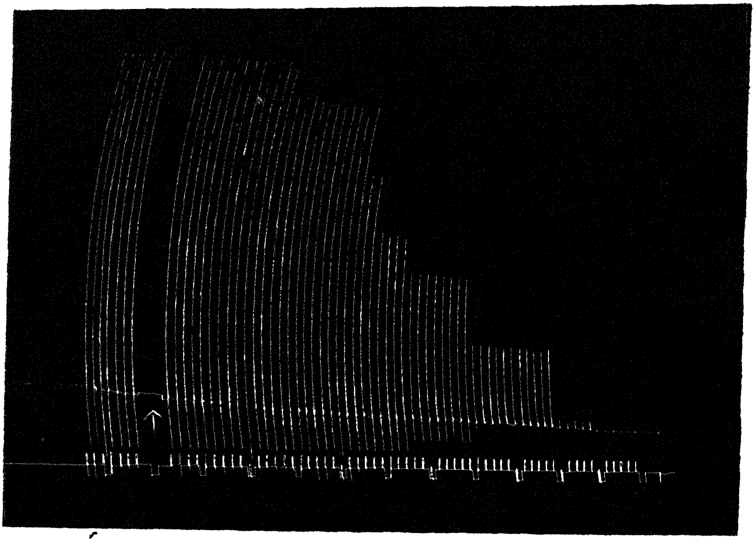

FIG. 2.—Onset of Block by a Dilute (<N/300) Solution of Amyl Stovaine in Tap-water Saline. Prior to the arrow the nerve bath contained normal saline.

In interpreting them, it must be borne in mind that the drugs are applied to the surface of the nerve, and that their rate of diffusion into the nerve trunk is slow (*v. supra*, p. 423). Hence the more superficial fibres of the nerve must be affected distinctly earlier than the deeper ones. This raises the question whether incomplete blockage of a nerve trunk is due to partial blockage of any of its individual fibres, or to complete blockage of a limited number of them.

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* In addition to the local anæsthetics with which this paper deals, we have also examined chloretone (tertiary trichlorbutyl alcohol) and yohimbine for the latter of which we are indebted to Dr. Waller. Both of these are credited with local anæsthetic properties.

In all our records, the muscular response, and therefore also the conductivity of the nerve, fails abruptly when it fails at all; either abruptly and rapidly as in fig. 1, or abruptly and slowly by a limited number of sharply defined, flat-topped steps, not exceeding eight in total. Marked instances of the latter are seen in figs. 2 and 3, as the effect of solutions that lead slowly to complete blockage of the nerve trunk.

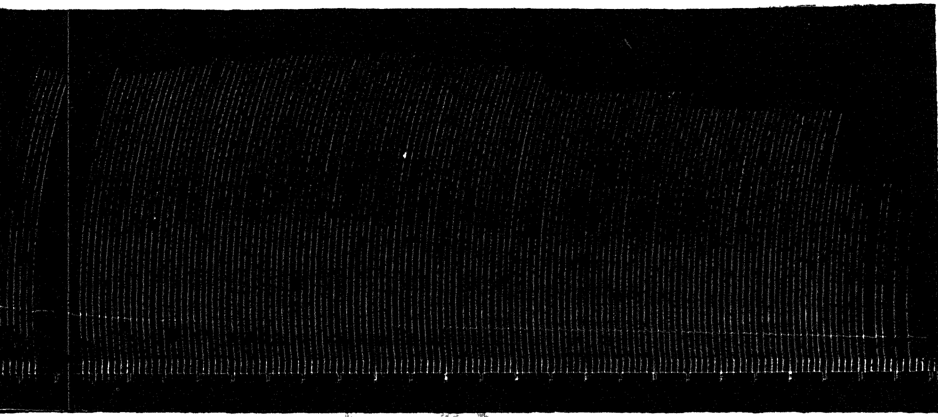

FIG. 3.—Block by a slowly acting Anæsthetic Solution. The drug, cocaine hydrochloride N/500 (= 0·07 per cent.), was applied at the white vertical line.

These do not represent the block in individual nerve fibres to be anywhere partial, but, as judged by the muscle, to be either complete or zero. For, on the one hand, the large number of small decrements in response that such partial blocking would necessarily entail is conspicuously absent, and, on the other hand, the strikingly small number and extreme flatness of the steps suffice to indicate that each one represents simultaneous complete blockage of large groups of fibres. The blockage of individual fibres is therefore commenced and completed within the interval (10 seconds)[*] between the stimuli.

Nor is partial blockage of individual fibres in evidence when the drug fails to produce complete[†] blockage of the nerve trunk.

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[*] A small number of observations with stimulation at intervals of 5 seconds yielded, without exception, records of the same type.

[†] A block was not regarded as complete until at least three consecutive stimuli evoked no response.

In such cases, when blockage is at all recognisable (fig. 4), abrupt irregularity replaces the abrupt regularity of figs. 2 and 3. In fig. 2, none of the steps is followed by any recovery during the drug bath, which, though slowly acting, is sufficiently concentrated to block with certainty. In fig. 3 the last three steps contain examples of reversion to the amplitude of the preceding; the concentration of the solution is just sufficient to ensure blockage. In fig. 4 not even the downward trend of the twitches is maintained. Here, after initial abrupt diminutions in the muscular response, only one stimulus (about 7 minutes after application of the drug) proved altogether ineffective, the succeeding stimuli evoking responses tending in general towards the normal amplitude (*cf.* Wedenski, *loc. cit.*), though interspersed with reversions towards failure.

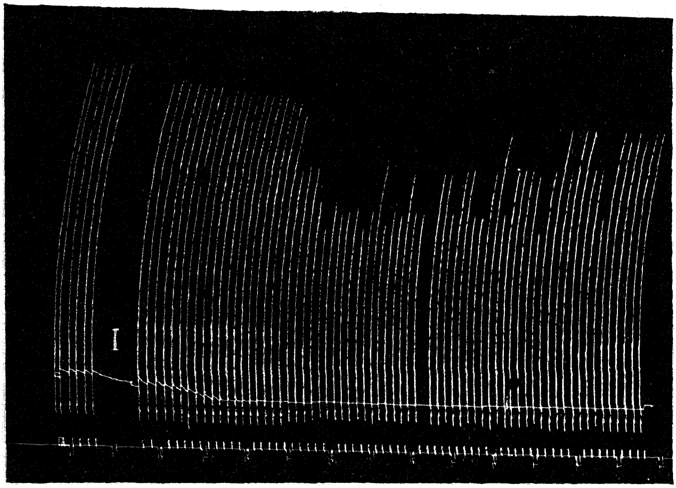

FIG. 4.—Failure to Produce Complete Block.
1. Application of drug, N/300 (= 0·1 per cent.) solution of cocaine base.

In short, even with the weakest solutions that are at all effective, *i.e.* with those most favourable to partial blockage of individual nerve fibres, there is, as judged by the muscle, no state of stable equilibrium between complete blockage of the fibre, on the one hand, and complete conductivity on the other.

These records recall observations by Grützner,* and by Gotch,† that stimulation of less than all the motor nerve fibres to a muscle leads to submaximal contraction, when similar stimulation of all of them produces maximal response. From this and other data Gotch urges that submaximal response in muscle, and in nerve, invariably implies maximal response of a limited number of fibres, rather than submaximal response of the whole (or of any) of them; in other words, that the response of individual fibres in both cases is, as in the heart, "all or nothing." In the case of muscle, this contention is endorsed by Keith Lucas* (*v. infra*). In the case of nerve, as judged by muscle, our observations appear to lend some support to the view.

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* Grützner, 'Arch. f. d. ges. Physiol., Bonn,' 1881, vol. 41, p. 256.

† Gotch, 'J. Physiol., Cambridge,' 1902, vol. 28, p. 395.

The relation of the anæsthetic block to the intensity of the stimulus has already been mentioned (*v. supra*, p. 433).

A block to response, evoked by a maximal stimulus, is complete for stimuli which (on the Berne scale) are several hundred times as intense, and this block only fails when the secondary coil is pushed up to the region where, in the absence of block, single induction shocks evoke the supermaximal twitches described by Fick.

It is doubtful that induction shocks of this intensity are in reality single stimuli. Whether so or not, they are noticeably injurious to the nerve (*cf.* Locke†), so far reducing its excitability that a normally maximal stimulus may for some minutes produce no response, and when at all, not then response of the original amplitude. Their effect cannot therefore be regarded as normal physiological excitation.

Considering, therefore, the failure of the local anæsthetics to reduce the amplitude of the nerve impulse without extinguishing the muscular response altogether, in conjunction with the markedly restricted (if existent) ability of increased (single) stimuli to evoke response through any anæsthetic block, it seems legitimate to conclude in agreement with Gotch that, apart from summation, the range of amplitude in the normal nerve impulse is exceedingly small—in short, that the response of individual fibres is substantially maximal or zero.

The records of recovery, when the drug solution is replaced by normal saline, are, *mutatis mutandis*, similar to those of failure of response.

The muscle twitch either returns early and grows rapidly and abruptly (after a weak anæsthetic bath), or later and by a more or less protracted series of abrupt steps (after a strong anæsthetic bath), as seen in fig. 1.

Records similar to the latter are described by Keith Lucas* as resulting from indirect stimulation of muscle, by single induction shocks, when these are gradually increased from subminimal to maximal. This step-growth in response is attributed by him to step-growth in the number of nerve fibres reached by the stimulus as it is increased in intensity, and his explanation is borne out by our records of slow restoration of the muscle twitch.

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* Keith Lucas, 'J. Physiol., Cambridge,' 1905—6, vol. 33, p. 125, and 1909, vol. 38, p. 125.

† F. S. Locke, *ibid.*, 1900—1901, vol. 26, p. xxxix ('Physiol. Soc. Proc.').

Since diffusion is slow (*supra*, p. 423), the superficial nerve fibres must recover from the effects of the drug appreciably earlier than the deeper ones, and since partial blocking of individual fibres is excluded, and the stimulus applied to the nerve trunk is constant, the step increments in response may fairly be assigned to step increments in the number of nerve fibres that conduct the nerve stimuli to the muscle.

It is of course obvious that, if (with constant total load) the number of muscle fibres that enter into contraction increases, the amplitude of contraction must increase, since the load per contracting fibre is correspondingly diminished.

VI. Conclusions.

1. Tested by their effect on the conductivity of frog's nerve, stovaine, its homologues, and Fourneau's new salt are more active local anæsthetics than is cocaine.

2. Methyl stovaine, amyl stovaine and Fourneau's salt appear to offer some particular advantage over the others.

3. Judged by muscular response, the anæsthetic block in individual nerve fibres, to impulses evoked by maximal single stimuli, throughout a wide range of intensity, is complete or zero.

4. This block is usually also,* within the same range, complete for impulses evoked by tetanising stimuli.

5. The block, when not complete for tetanising stimuli, is largely resistant to summation (*cf.* Wedenski).

6. From the effects of the local anæsthetics in question, it appears that the amplitude of a motor nerve impulse is, within normal limits of stimulation, substantially maximal or zero (*cf.* Gotch).

7. *Ceteris paribus*, the amplitude of a muscle twitch depends on the number of fibres involved (*cf.* Grützner, Gotch, and Keith Lucas).

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* *Vide* pp. 423, 424.

Carbon Dioxide Output during Decerebrate Rigidity.
(Preliminary Communication.)

By H. E. Roaf, D.Sc. Liverpool, M.D. Toronto.

(Communicated by Prof. C. S. Sherrington, F.R.S. Received December 28, 1910, —Read March 2, 1911.)

(From the Physiological Laboratory, University of Liverpool.)

These experiments were undertaken, at suggestion of Prof. Sherrington, to attempt a determination of the amount of energy necessary for maintaining tonus in skeletal muscle.

The carbon dioxide output has been measured in decerebrate cats, and its amount compared with the amount given off by the same animal after procedures that abolish the tonus of the decerebrate preparation. These procedures have consisted of decapitation and intravenous injection of curare, The pulmonary ventilation was uniform throughout, as artificial respiration was carried out by means of a pump.

The animals were anæsthetised and decerebrated. They were kept in a chamber, the air of which was moist and approximately at body temperature. The ingoing air was freed from carbon dioxide, moistened and warmed. The outgoing air was dried, and the carbon dioxide was absorbed by soda lime and weighed. The period of experiment was divided into two halves of two hours' duration each. At the end of the first half the observations were interrupted for just so long as was necessary for the procedures required for the second half.

The results are expressed as a ratio, namely, the average percentage that the carbon dioxide output of the second half forms of the first.

Decapitation lowered the carbon dioxide output to 66·9 per cent. (four experiments), the output in the previous two hours with the decerebrate preparation being considered as 100. The diminished output is mainly due to lowered metabolism of skeletal muscle, because in an experiment in which the cat had been eviscerated previous to the respiration experiment the second half gave a result 61·1 per cent. of the first.

It was thought possible that the diminished output after decapitation might be due to lowered blood-pressure, and cutting the splanchnic nerves on both sides did reduce the output to 90·7 per cent. (two experiments). The greater reduction in the decapitate experiments cannot be due to hæmorrhage, as practically no blood was lost by decapitation.

When the rigidity was abolished by curare the carbon dioxide output amounted to 100·8 per cent. (four experiments).

Controls.—As a guarantee of the correct working of the method during the two hours following decerebration, it may be stated that during a second period with the same decerebrate preparation the carbon dioxide output was 94·3 per cent. of the first period (two experiments).

Although precautions were taken to maintain a constant body temperature it varied a little during some of the experiments. If the "temperature coefficient" of carbon dioxide output be taken as lying between 2 and 3 for each rise of 10° C., the fall of temperature practically accounts for the diminution of carbon dioxide output in the control and splanchnic, but not in the decapitate experiments. In two of the curare experiments the internal temperature rose, and the carbon dioxide output also went up, thus the average shows no difference from the non-curarised condition.

These experiments indicate: (1) That the abolition of decerebrate rigidity by curare does not lower the carbon dioxide output, and hence the postural tonus requires very little expenditure of energy. Parnas* has recently shown that maintained contraction of the adductor muscles of certain bivalves requires much less energy than the holding up of the same weight by vertebrate skeletal muscle.

(2) That decapitation causes a marked fall in carbon dioxide output, which is not due to the abolition of tonus or fall of blood-pressure.

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* 'Arch. f. d. ges. Physiol.,' 1910, vol. 134, p. 441.

Reversal of the Reflex Effect of an Afferent Nerve by Altering the Character of the Electrical Stimulus Applied.

By C. S. SHERRINGTON, F.R.S., and S. C. M. SOWTON.

(From the Physiological Laboratory, University of Liverpool.)

(Received December 30, 1910,—Read March 2, 1911.)

Stimulation of an afferent limb-nerve in the decerebrate or decapitate mammal (cat, dog) gives as its reflex result flexion of that limb; during this flexion the pure extensor muscles of the limb relax under central inhibition. In the hind limb this reflex effect is observable in the isolated vasto-crureus, the main extensor muscle of the knee; that muscle if engaged in contraction relaxes* when the afferent nerve is stimulated. Its elongation is the sign of the central inhibition which takes place. If the reflex stimulation be strong the muscle relaxes quickly and greatly; if the stimulation be weak the relaxation is slower and less ample. These results are easily demonstrable by using as a stimulus either faradism or mechanical stimulation such as ligation of the central stump of the afferent nerve.

Usual and uniform as this result is, we find it possible in the decerebrate preparation under certain conditions to obtain reflex contraction of vasto-crureus as well as reflex inhibition, and to elicit the contraction through the same afferent nerves as under other conditions so regularly elicit inhibition. The conditions influencing the nature of the reflex result in this respect are (1) the strength and (2) the form of the electrical stimulus applied to the afferent nerve, and (3) the reflex state obtaining in the preparation at the time.

The reflex preparation used in our observations is the same as that employed by one of us previously† for the examination of extensor reflexes in the limb. The animal, after decerebration under profound chloroform narcosis, has the vasto-crureus muscle of one limb isolated by severance of all other nerves than its own in the whole limb and by actual resection of the flexors and extensors of the hip. The vasto-crureus thus remains the only unparalysed muscle in the limb, and its natural attachments and nerve and blood supply are undisturbed. The femur is securely fixed in an appropriate metal holder. The preparation lies supine on a warmed table. A thread attached below the knee connects the limb with the myograph lever. The afferent nerve to be stimulated is carefully separated for as long a length as possible, and the electrical stimuli applied to it are delivered by non-polarisable clay electrodes of the du Bois Reymond pattern, slung from the ceiling above.

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* Sherrington, 'Roy. Soc. Proc.,' 1893, vol. 53, p. 407.

† Sherrington, 'Quart. Journ. Exp. Physiol.,' 1909, vol. 2, p. 115.

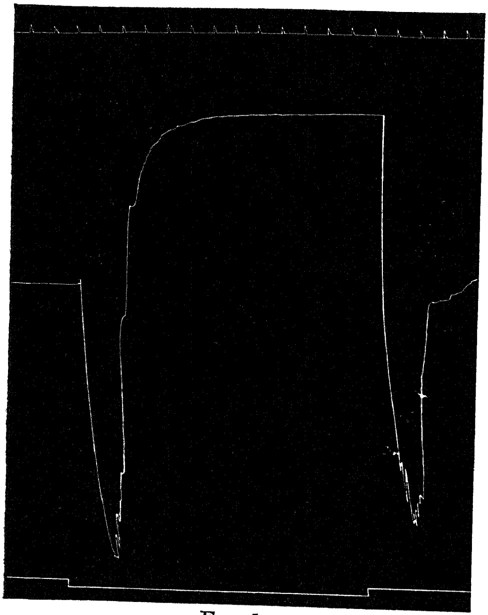

Fig. 1.

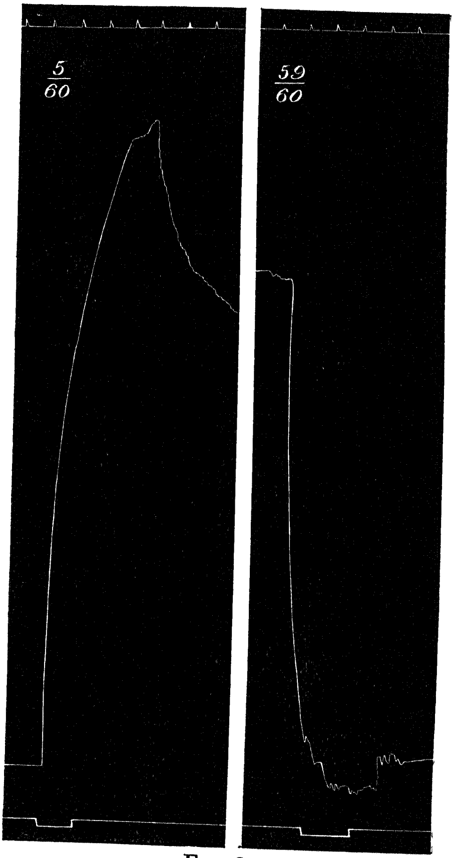

Fig. 2.

Fig. 1.—Isolated Vasto-crureus Muscle ; decerebrate preparation. Galvanic current (strong, ascending), applied to ipsilateral afferent (popliteal) nerve ; electrodes non-polarisable. Time above in seconds ; signal below. Descent of the myogram line means relaxation ; ascent means contraction. The make of the current evokes a marked inhibitory relaxation of the muscle ; this is followed by a rebound with heightened tonus during the passage of the current. The current is then cut off, and the break excites a second marked inhibitory relaxation of the muscle.

Fig. 2.—Isolated Vasto-crureus Muscle ; decerebrate preparation. Ascent of the myogram line means contraction of the muscle ; descent means inhibitory relaxation. Time above in seconds ; signal below. During the period marked by the downward notch in the signal line a series of brief galvanic currents, each lasting 0·04 second and succeeding each other at the rate of 12 per second, were delivered in the ascending direction through the central stump of the severed ipsilateral popliteal nerve. Both records are from the same experiment with only a few minutes' interval between them. The strength of current was quite weak in the left-hand record (5 cm. divisions of 60 cm. platinum-iridium wire rheochord used as a potentiometer), and was quite strong in the right-hand record (59 cm. divisions of the rheocord similarly used). The weak stimulus evokes reflex contraction ; the stronger stimulus reflex inhibition.

I. Intensity and form of electrical stimulation.

With all forms of electric stimulus employed by us the stimulation, if it is to produce reflex contraction of the extensor muscle, must not be intense. This point is best dealt with when considering the influence of the form of the electric stimulus on the reflex result. As to this latter determinant factor, namely, the form of the electrical stimulus, our experience is that galvanic currents yield reflex contraction more readily than do faradic.

(α) A constant galvanic current applied to the afferent nerve evokes reflex effect both at make and break. With ordinary strengths of current the result resembles the well-known du Bois Reymond formula for the effect of the current on the ordinary muscle-nerve preparation, inasmuch as there is obvious excitation of the nerve at make and break and not during the continued passage of the current. With the reflex preparation above described the effect at make and break when the current is of moderate or high strength is inhibition (fig. 1); when the current is quite weak the reflex effect may often be distinct though weak contraction both at make and break. Current direction and current strength and duration influence the du Bois Reymond formula in the reflex preparation as they do in the simple nerve-muscle preparation: we have studied these variations, but apart from the above general result they bear little on the problem at hand, and we defer them for a fuller account.

(β) Under stimulation of the afferent nerve by series of brief galvanic currents, ascending or descending, the reflex result on the tonic extensor muscle is in most cases contraction when the currents are quite weak, and is always inhibitory relaxation when the currents are quite strong (fig. 2). With intermediate strengths the result is a complex of the above; there is initial contraction, passing over into inhibition as the stimulus continues. The duration of the initial contraction is less, and the supervention of inhibition earlier as the intensity of the stimulus is progressively increased in successive stimulations. Even with weak stimulation the initial contraction tends under prolonged application of the stimulus to give way and pass into inhibitory relaxation.

(γ) With faradic stimulation from the ordinary du Bois Reymond inductorium the results obtained are (fig. 3) somewhat similar to those just mentioned for galvanic currents. Weak stimulation tends to give some contraction, while moderate and strong stimulation always produces inhibitory relaxation. With the induced currents, however, the contraction is less readily developed than with the galvanic, and passes over into inhibition more speedily; moreover, the contraction when obtained is more abrupt in onset and disappearance. With both forms of current, however,

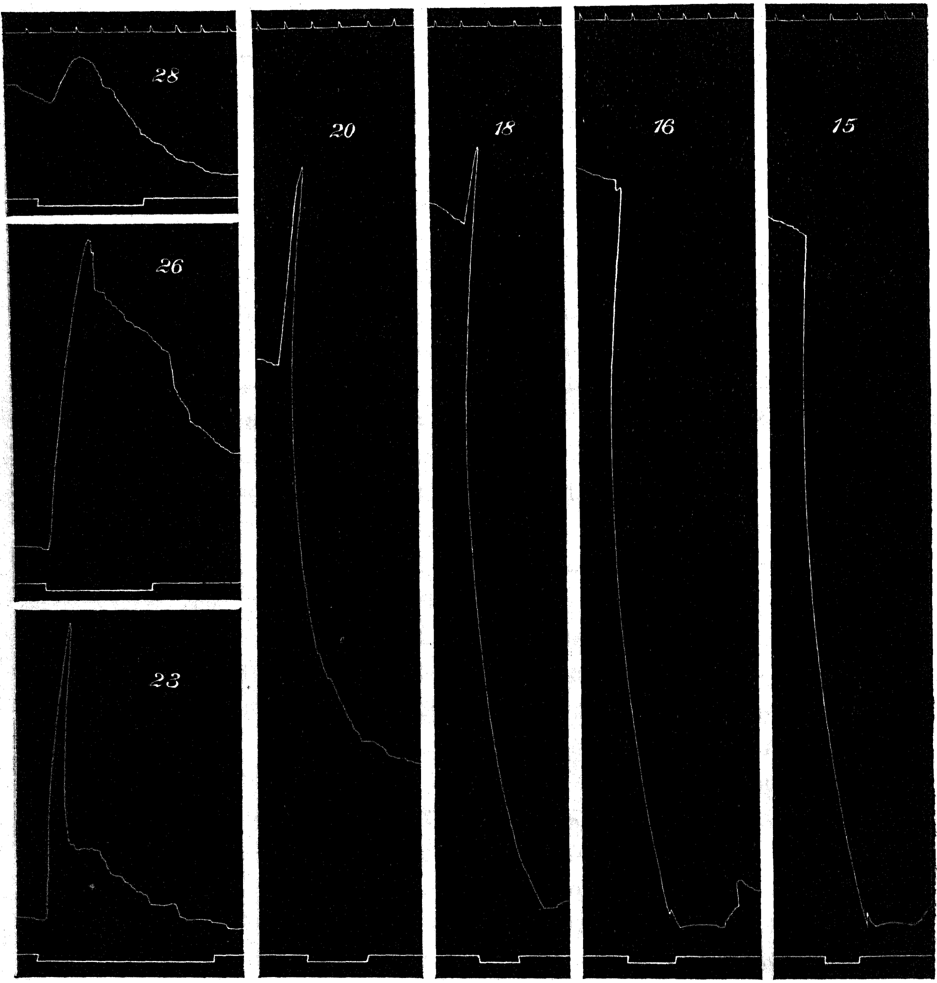

Fig. 3.—Isolated Vasto-crureus Muscle; decerebrate preparation. Ascent of myogram lever signifies contraction; descent inhibitory relaxation. Time above in seconds; signal below. The series of records were all obtained in succession from the same preparation. During the downward gap in the signal line series of double induction shocks at the rate of 28 per second from an ordinary inductorium were delivered through non-polarisable electrodes to the central stump of the severed ipsilateral popliteal nerve. The series of induced currents were successively increased in strength by bringing the secondary coil from 28 cm. to 26 cm., 23 cm., 20 cm., 18 cm., 16 cm., and finally 15 cm. distance from the primary. With this increase of intensity of stimulus the reflex effect on the muscle changes from contraction to inhibitory relaxation.

a stronger stimulus elicits a more abrupt contraction than does a weaker, and this has to be remembered in comparing particular instances of the two kinds of stimulation. With the faradic stimulus especially the contraction phase of the reflex often presents itself on the record as a mere spike ushering in and immediately followed by swift inhibitory relaxation. With faradic stimulation of moderate or strong intensity all trace of contraction usually disappears.

(δ) The difference between the effect of galvanic and faradic stimulation might obviously be referable to the more abrupt and momentary character of the latter. From our observations we received the impression that with stimuli still less abrupt than the make and break of the constant current as ordinarily performed, the contraction phase of the reflexes might be better developed. As a means for providing such stimuli we turned to the rotating rheonome of v. Kries. A description of this instrument is furnished by Metzner* (1893), who used it for stimulation of the frog nerve-muscle preparation in his research on work and heat output in muscle. It is essentially a key which rhythmically alternates the direction and varies by rectilinear increments and decrements the amount of galvanic current delivered through the stimulating electrodes. With two small modifications we have employed it in its original form, and with the slope of increment and decrement of current at less than the maximal steepness which the instrument permits. For comparing its effects on the preparation with those of faradic stimulation from the ordinary inductorium, we arranged our apparatus in the manner shown in the figure subjoined (fig. 4). The circuit was fed by one Leclanché cell. The speed of rotation generally used for the rheonome gave 20 stimuli per second. The electrodes were non-polarisable, of the du Bois Reymond pattern, and the distance between them on the afferent nerve was always about 1 cm.

Like the serial brief galvanic currents simply made and broken, the galvanic stimulation given by the rotating rheonome produces reflex inhibition when the current is strong. But when the current is weak it produces reflex contraction, and as was anticipated the reflex contraction is more pronounced and more durable than with the other forms of stimulation we have used. By employing it we find reflex contractions of the extensor muscle obtainable which exhibit pre-eminently certain characteristic features. Among these features are peculiar slowness of development of the contraction, mild and steady intensity of contraction, long maintenance of the contraction without decline or interruption by fatigue or tremor, and slow subsidence of the contraction on terminating the stimulation (fig. 5). In fact with the stimulus which the rotating rheonome provides, we obtain extensor contraction reflexes of a characteristic kind.

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* Rudolf Metzner, 'Archiv f. Physiologie,' 1893, Supplement-Band, p. 84.

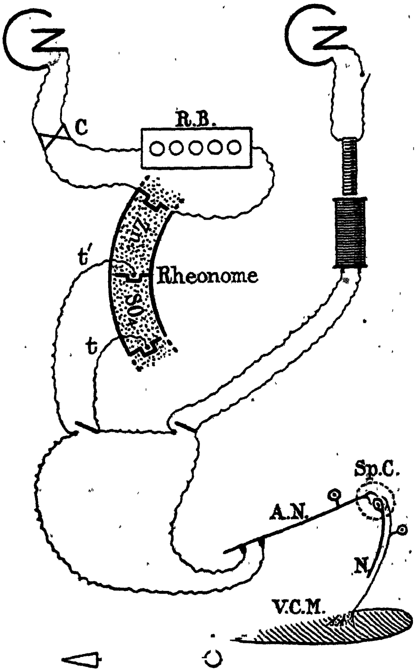

FIG. 4.—Arrangement of Apparatus for Comparing Effect of Galvanic Stimulation by Rotating Rheonome with that of Faradisation from ordinary Inductorium. *C.*, commutator in galvanic circuit; *R.B.*, resistance box; $ZnSO_4$, saturated zinc sulphate solution in trough of rotating rheonome; *t* and *t'*, travelling electrodes in trough in rotating rheonome. Rheonome, rotating rheonome, only a small arc of the complete circle being indicated. *A.N.*, afferent nerve with non-polarisable electrodes applied; *Sp.C.*, spinal cord; *N.*, nerve of vasto-crureus muscle; *V.C.M.*, vasto-crureus muscle with tendon attached to myograph.

They differ from the reflexes usually resulting from weak stimuli in several ways. With weak stimuli of other kinds it is usual for the reflex contraction to decline soon, even during the continuance of the stimulation, in one or other of two ways. (1) Either there ensues "fatigue," which though slight is yet sufficient to cancel soon the excitation of a stimulus which is itself but little above threshold value. This is the phenomenon that accounts for the, at first sight, paradoxical result that the reflex contraction evoked by a quite weak stimulus tends to tire out sooner than that evoked by a strong stimulus. Fig. 6 exemplifies this result in reflex contractions of a flexor muscle evoked by faradic stimulation of the afferent nerve,* (2) Another way in which decline of a weak reflex contraction ensues rapidly during continuance of the stimulus is, in the extensor, decerebrate preparation, by supervention of reflex inhibitory relaxation, the character of the reflex result

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* *Cf.* also Sherrington, 'Integrative Action of the Nervous System,' 1906, p. 219.

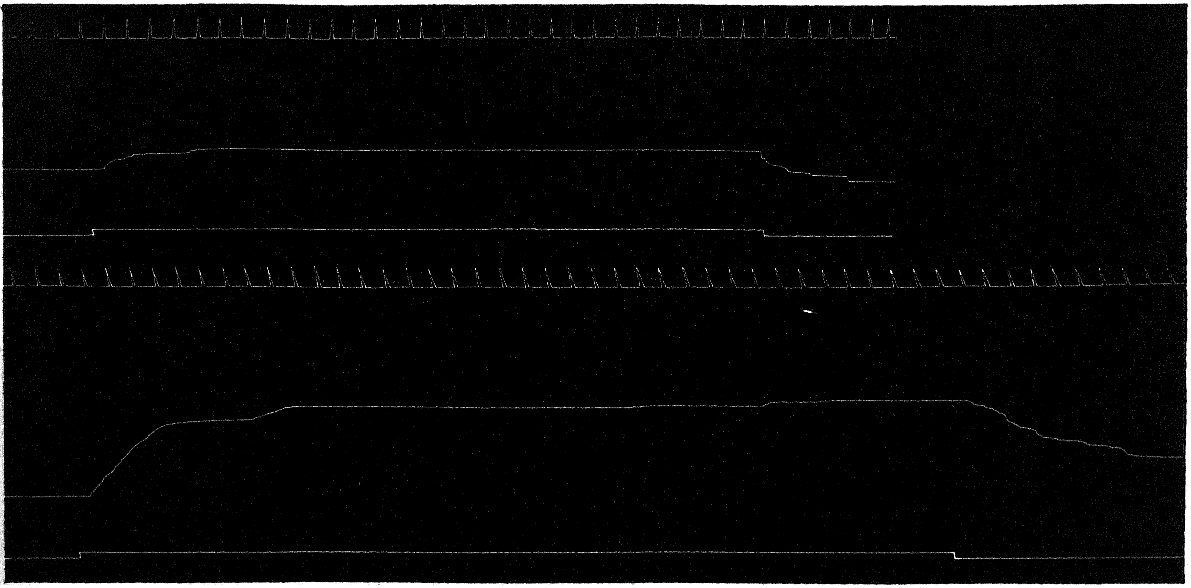

Fig. 5.—Isolated Vasto-crureus Muscle; decerebrate preparation. Tonus-like reflexes. Ascent of the myogram line signifies contraction. Time in seconds above; signal below. Throughout the period in which the signal line is heightened a series of brief galvanic currents alternating in direction and alternately waxing and waning in current strength was delivered at the rate of about 22 per second *viâ* non-polarisable electrodes to the central stump of severed ipsilateral popliteal nerve. The scale of magnification by the myograph lever is the same as in the other tracings.

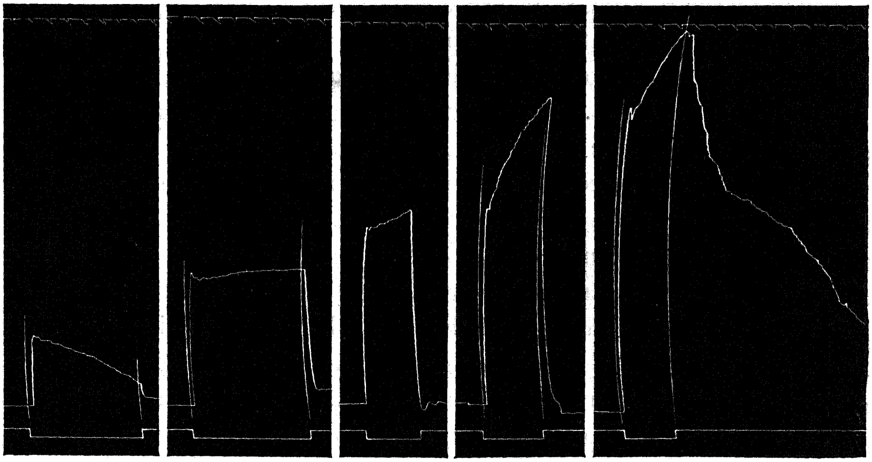

Fig. 6.—Isolated Tensor Fasciæ Femoris Muscle; decapitate preparation. Time above in seconds; signal below. During the downward gaps in the signal line the popliteal nerve, central end, was stimulated by faradism, and in the successive records the strength of the stimulation was successively increased. The reflexes form a series of reflex contractions of successively increasing intensity. With the weakest stimulus, the intensity of the contraction declines during the 6 seconds' continuance of the stimulus; with the next stronger stimulus, the intensity of contraction is maintained during the 6 seconds' continuance of the stimulus; with the still stronger stimuli, the intensity of contraction increases during the stimulus; and with the strongest stimulus, the contraction persists in some measure as after discharge for several seconds subsequent to cessation of the stimulus itself.

changing from contraction to inhibition as the stimulation proceeds. Of this examples have been already furnished above (fig. 3). The weak reflex contractions of the extensor evoked by mild intensities of stimulation, very little above threshold value, given by the rotating rheonome, show little and sometimes nothing of either of the above forms of decline. Nor is their long duration merely simulated by the intervention of after-tonus supporting them in the way it supports shortenings of the muscle in virtue of the plasticity of the tonic muscle.* This possibility is negatived by the fact that on cessation of the stimulus the contraction at once, though gradually, subsides (fig. 5).

II. Apart from intensity and form of the electric stimulus, a further condition essential for eliciting extensor contraction-reflexes from afferents of the selfsame limb is the absence of "shock" in the preparation. These ipsilateral reflex contractions are, in our experience, unobtainable in the decapitate preparation. Obtainable in the decerebrate preparation by the above-described means when the muscle exhibits good reflex tonus, they are poor or inelicitable if the tonus is imperfect. It appears to us that conditions which impede the development of the extensor tonus, or set that tonus aside, similarly impede or set aside these ipsilateral reflex contractions of the extensor. The association thus evident between these reflex contractions and the reflex tonus, together with the features of the contractions as elicited by the rotating rheonome and the resemblance they bear to reflex tonus itself, suggests that these contraction reflexes are essentially tonus reflexes. The incidence of these reflexes and of the tonus is upon the very same muscles of the limb. We think that, especially as elicited by the weak galvanic stimuli from the rotating rheonome, these reflex contractions are in fact to be regarded as accessions of tonus, the reflex outcome of the electric stimulation.

To regard them as tonus reflexes seems justified further by outstanding forms which these reflexes sometimes assume. Fig. 7 exemplifies such a case. On applying to ipsilateral popliteal the weak galvanic stimulation through the v. Kries rheonome, the knee-extensor began to slowly shorten, its tonic length continuing to shorten throughout the 30 seconds during which the same unaltered stimulation was in progress. On discontinuing the stimulation the muscle remained of the length to which the electrically-induced tonus reflex had brought it; this after-maintenance being the result of the plasticity of the preparation ("shortening reaction").† Eighteen seconds later the same afferent nerve as that which under the galvanic rheonome stimulus had elicited the tonus reflex of contraction was stimulated through the same electrodes as before with weak faradic currents (60 Kronecker Berne units) for one second. The result of this latter stimulation is seen to be immediate reflex inhibition. By these two different stimuli two diametrically opposite reflex effects are therefore elicitable from the same muscle by the same electrodes acting on the same afferent nerve, and at a few seconds' interval between the two reactions.

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* Sherrington, 'Quart. Journ. Exp. Physiol.,' 1909, vol. 2, p. 109.

† Sherrington, 'Quart. Journ. Exp. Physiol.,' 1909, vol. 2.

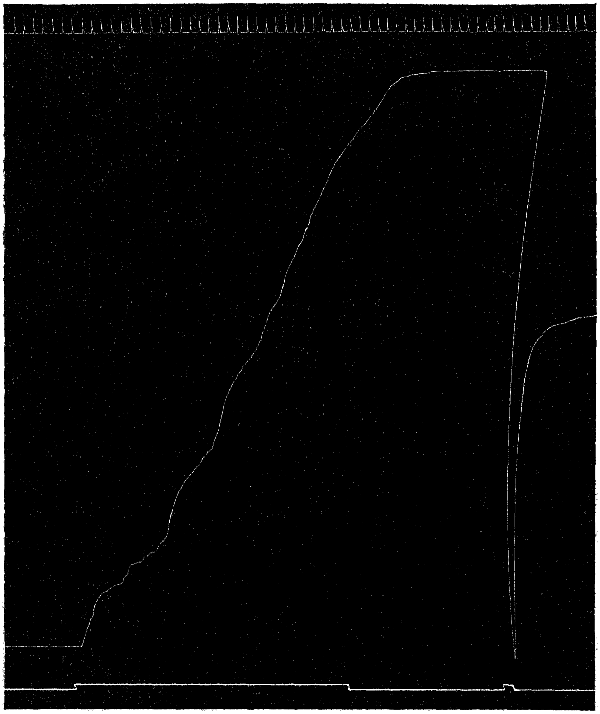

FIG. 7.—Isolated Vasto-crureus Muscle; decerebrate preparation. Ascent of the myogram line indicates shortening of the muscle; descent indicates lengthening. Time above in seconds; signal below, moves upward to indicate stimulation. Two periods of stimulation are shown by the signal: an earlier more prolonged period, and a later shorter one. During the earlier the popliteal nerve (central stump) was stimulated by weak galvanic currents provided by the rotating rheonome, and in consequence of this stimulation the tonus of the muscle rose. After this stimulation the afferent nerve was, through the same electrodes, stimulated by weak faradisation for 1 second; inhibitory relaxation immediately resulted.

A comparable result to that just mentioned is obtainable from the afferent nerve, *e.g.*, n. popliteus, by placing two pairs of electrodes upon it, and stimulating by faradism with one pair, by weak galvanic currents from the rheonome with the other pair. It makes no difference which of the electrodes are proximal or distal on the nerve-trunk. If while the galvanic stimulation is eliciting a tonus reflex the faradic stimulus be applied, there results immediate reflex inhibition, which is recovered from when the faradic stimulus is withdrawn.

It would appear, therefore, that the currents provided by the rotating rheonome act, when not too strong, as stimuli capable of exciting reflex tonus-contractions in the tonic extensor muscles of the decerebrate preparation. As to the actual character of the stimulus which is the source of the natural reflex tonus, *e.g.* in the decerebrate preparation, there is still little knowledge, except that it must be regarded as probably mechanical in kind. The fact that tonus reflexes not far dissimilar in outward features from natural tonus are obtainable by the above-described intermittent electric stimulation of the bared afferent nerve suggests that in the natural tonus itself the reaction of the afferent nerve is essentially rhythmic and intermittent, though the stimulus playing on its receptors may be constant, as is also the final mechanical result exhibited by the reacting muscle.

There is much evidence in favour of the reflex extensor tonus of the decerebrate preparation being postural in meaning,* of that tonus being in fact the postural reflex executive of standing. In this connection it is interesting to note that a contralateral reflex usually accompanies the ipsilateral extensor contraction reflex above described, and that this contralateral reflex is, like the ipsilateral, a reflex extension of the limb. In this respect these ipsilateral extension reflexes provoked by electric stimuli resemble the ipsilateral extension reflex (the "extensor thrust") provocable by mechanical pressure on the planta. The fact that the tonic extension reflex elicited by weak galvanic currents is accompanied by concurrent extensor contraction in the contralateral fellow limb argues that the former reflex (ipsilateral extension) is not a part of the limb's stepping reflex, because in stepping the two fellow limbs are always conversely instead of symmetrically moved.

The afferent limb-nerves from which we have been able, by appropriate means, to elicit the reflex contraction of the ipsilateral extensor have been popliteal, plantar, peroneal, internal saphenous, obturator, and hamstring. We have found it more difficult to obtain the reflex from the hamstring nerve than from any of the others, and from the peroneal more difficult than from the popliteal.

From these afferent nerves, therefore, two different reflex effects upon the tonic extensor muscle are obtainable, according as the stimulus employed for the afferent is weak or strong, &c. The case recalls the double effect obtainable from the opening muscle of the *Astacus* claw preparation;[*] also the difference of result of infrequent, as compared with frequent, stimuli applied to the distal sciatic nerve preparation when the blood-volume[†] of the limb is observed—inhibitory dilatation resulting from the former, vascular contraction and constriction from the latter, and mixed results with intermediate rates. F. W. Fröhlich[‡] finds certain afferent nerves in the frog yield sometimes reflex excitation, sometimes reflex inhibition, according to the conditions of stimulus employed upon them.

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* Sherrington, 'Journ. Physiol.,' 1910, vol. 40.

The case differs from the reversal of reflex effect studied by v. Uexkull,[§] and by R. Magnus,[||] in the circumstance that in their reversals the stimulus-strength, etc., remain the same, and the changed condition is a central (R. Magnus)[¶] change brought about by alteration of posture, etc. Change of posture at the knee in the isolated vasto-crureus preparation does not indubitably influence the reversal we are dealing with in this paper. Thus, whether the knee is fully flexed, or fully extended, at the time when the stimulus is delivered, does not in our case clearly influence the reflex result (fig. 8); that result remains practically the same whatever the initial posture of the knee, *i.e.* whatever the tonic lengths obtaining at the time in vasto-crureus.

The case differs also from the reversal of reflex effect brought about by strychnine,[**] inasmuch as in the latter not only is no change in the intensity or mode of stimulation required, but also none in the posture, etc., of the reacting preparation.

It is obvious that one way of accounting for the difference of reflex effect produced by varying the intensity and form of the stimulation of the afferent nerve is to assume the co-existence in the nerve of two different kinds of afferent nerve-fibres, exerting diametrically opposed reflex effects on the extensor muscle, and to suppose that of these nerve-fibres one kind is better excited by weak inabrupt stimulation, and the other kind by abrupt and relatively intense stimulation. It appears to us premature, however, to accept unreservedly this hypothesis as a solution of the problem.

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[*] Richet, 'Archives de Physiologie,' 1879, Paris; Biedermann, 'Sitzungsb. d. k. k. Akad. d. Wiss.,' Wien, 1887; Piotrowski; Fr. Fröhlich, 'Zeits. f. allgem. Physiol.,' 1908, vol. 7.

[†] Bowditch and Warren, 'Journ. Physiol.,' 1886, vol. 7, p. 32.

[‡] 'Zeits. f. allg. Physiol.,' 1909, vol. 9, p. 85; also A. Tiedemann, *ibid.*, 1910, vol. 10, p. 196.

[§] 'Zeitschr. f. Biol.,' 1897, vol. 35.

[||] 'Pflüger's Archiv,' 1910, vol. 125.

[¶] *Ibid.*

[**] Sherrington, 'Roy. Soc. Proc.,' B, 1905, vol. 76.

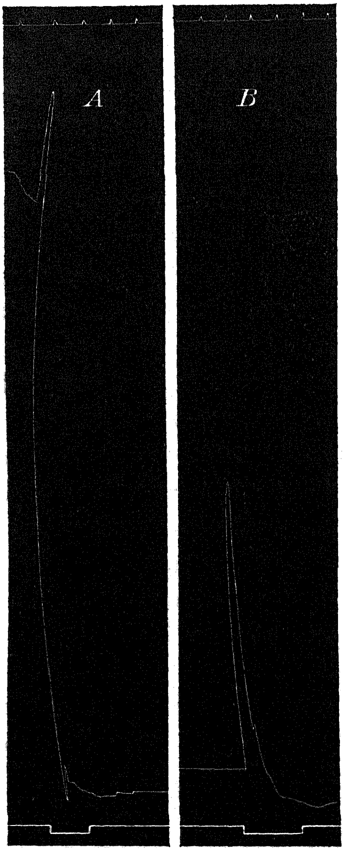

FIG. 8.—Isolated Vasto-crureus Muscle; decerebrate preparation. The nerve was weakly faradised by the same current through the same electrodes in both *A* and *B*, and with only a few seconds' interval between the two stimulations. The only change in the conditions was that in *A* the knee at outset of the observation starts almost fully extended, whereas in *B* it is almost fully flexed. The reflex result in both cases is a transitory initial reflex contraction followed by a very complete inhibitory relaxation. Time above in seconds; signal below.

Experimental Studies in Indian Cottons.[*]

By H. Martin Leake, M.A., Economic Botanist to the Government of the United Provinces of Agra and Oudh, India.

(Communicated by W. Bateson, F.R.S. Received January 9,—Read March 2, 1911.)

In the course of these experiments, most of the types of cotton cultivated in India have been grown, but the experiments are confined, with few exceptions, to those types common to Northern India. These types fall into two groups, which are characterised by the form of the secondary branches. These may be either monopodial or sympodial,[†] and in pure types they are either monopodial, with a few apical branches sympodial, or sympodial, with a few basal branches monopodial. Types in which the secondary branches are all monopodial or all sympodial do not appear to occur, though individuals may be so characterised. The types which have given the material for these experiments have been grown in pure culture for a series of years, and may be grouped as follows:—[‡]

Monopodial Types.[§]

(*a*) Foliage green—

Plant glabrous or nearly so, branches ascending, bracteoles triangular with margin entire or dentate, leaf factor < 2. Type 1, *G. obtusifolium*, Roxb.

Plant pubescent, branches spreading, bracteoles deeply auriculate or reniform with margin deeply serrate, leaf factor < 2. Type 2, *G. herbaceum*, Linn.

(*b*) Foliage red—

Plant differing from Type 1 in colour of foliage and petals, and in leaf factor, which is > 3. Type 3, *G. arboreum*, Linn.

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[*] Conducted at Cawnpore by the Botanical Research Section of the U. P. Department of Agriculture.

[†] Balls, 'Journ. Ag. Soc.,' vol. 2, p. 336; Cook, U.S. Dept. of Ag., Bureau of Plant Industry, Bull. 88.

[‡] *Cf.* the classifications of Todaro, 'Rel. sul. Cult. dei Cotoni,' 1877; Watt, 'The Wild and Cultivated Cotton Plants of the World'; Gammie, 'The Indian Cottons.' In all these the habit of the plant is not considered.

[§] Only sufficient description is here given to serve the purposes of the present note.

Sympodial Types.

(*a*) Foliage green—

  (1) Leaf factor < 2—

    i. Leaf commonly with three lobes, bracteoles small, closely enveloping bud and fruit; petals yellow. Type 4, *G. indicum*, Lamk.
    ii. Leaf lobes 5—7, petals yellow. Type 5.*
    iii. Leaf lobes 5—7, petals white. Types 6 and 7.

  (2) Leaf factor > 3—

    i. Leaf lobes 5—7, petals yellow. Type 8.
    ii. Leaf lobes 5—7, petals white. Type 9.
    iii. Bracteoles large, continuing to grow during development of boll, which is large, many seeded; petals pale yellow. Type 10, *G. cernuum*, Tod.

(*b*) Foliage red—

  Leaf factor > 3, petal red on white. Type 11, *G. sanguineum*, Hassk., var. *minor* of Gammie.

The experiments include observations on the following characters: (1) colour of corolla; (2) red colouring matter of sap; (3) leaf factor; (4) type of branching and length of vegetative period; (5) glands of the leaf.

1. *Colour of the Corolla.*—This may be either yellow or white. The heterozygous form has a full yellow petal—which colour is, therefore, completely dominant—and gives in subsequent generations pure yellows (DD), impure yellows (DR), and whites (RR), the latter giving whites only. The number of individuals here handled are too few to give reliable numerical results.

2. *Red Colouring Matter of the Sap.*—The presence of a red colouring matter in the sap, such as occurs in Type 3 and Type 11, is dominant to its absence. The dominance is, however, incomplete, and the heterozygous and pure forms are readily distinguished from each other by the depth of colour. From a cross between Type 3 (red flowered) and Type 9 (white flowered) it has been found possible to isolate pure red on white flowered (*cf.* Type 11) and pure yellow flowered (*cf.* Type 8) forms. Type 3 must therefore be considered to bear the yellow colour, masked by the red sap colour, which is superimposed. The Mendelian expectation in the F_2 generation (on the assumption that Type 3 possesses the two dominant characters, yellow petal

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* Types 5—9 are included in the *G. neglectum* and *G. roseum* of Todaro.

and red sap colour, which are absent in Type 9) is found to hold within a fair degree of approximation. The correctness of the supposition here made is shown by the behaviour of a cross between Type 3 and Type 4. In this case the yellow character is present in both types, and a simple pair of allelomorphic characters is under consideration.

3. *Leaf Factor.*—The degree of incision of the leaf lobe has formed the basis of most classifications of the Indian cottons. This character has been found capable of a numerical expression, to which the term leaf factor has been applied by the author.* This character of the leaf—a multiple organ—is, within limits, a plant character also, and may be fully defined as the average factor of all leaves arising from the monopodial branches of the plant.

Pure types have been found to fall into two groups—a broad-lobed, with a leaf factor < 2, and a narrow-lobed, with a leaf factor > 3. In no case has purity of this character been found in the offspring of a plant with a leaf factor intermediate between these two values.

When crossing occurs between a broad- and a narrow-lobed type, the heterozygote has a leaf factor which is approximately the arithmetic mean of those of the two parental types. In the F_2 generation a series of forms is obtained, in which every value of leaf factor is found. The distribution of individuals is, however, not uniform, but shows a definite arrangement into three groups, whose mean values agree approximately with those of the two parental types and the F_2 heterozygote. The curve obtained by plotting such a series is trimodal, the three modes corresponding respectively to the two parental and the intermediate values. In subsequent generations the average leaf factor of the offspring approximates to that of the parent. In other words, narrow- and broad-lobed plants will be found to breed pure, while the intermediate forms, though they may give a series of individuals with varying values of leaf factor, will give a larger proportion of broad- or narrow-lobed offspring, according as they themselves possess a leaf factor approximating to the broad- or to the narrow-lobed types.

4. *Type of Branching and Length of Vegetative Period.*—As has been noted, the secondary branches of the cotton plant may be either monopodial or sympodial, and in some types the secondary branches are, with the exception of a few of the basal ones, all sympodial, while in others, with the exception of only a few apical branches, they are all monopodial. In the latter case the vegetative period is prolonged, and no fruit is formed until the tertiary branches are developed. The type of branching, therefore, can be recorded in two ways, either by the fraction of the main stem, measured from the

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* 'Journ. As. Soc. Bengal,' vol. 4, p. 13.

base, bearing monopodial secondary branches, or by the length of the vegetative period, recorded in days from the time of sowing to the date of appearance of the first flower. Both methods are subject to secondary influences which render the record approximate only. The inter-relation between the two methods of record is given by the coefficient of correlation, which was found to be 0·681 for a series of plants forming the F_2 generation of a single cross.

When a monopodial and a sympodial type are crossed, the heterozygote, though intermediate, approximates to the sympodial parent. In the F_2 generation a series of forms is obtained, of some of which the vegetative period is as short as, or shorter than, that of the sympodial parent, while it rarely reaches the length of the full monopodial parent. On plotting the curve of such a series a single mode only appears, whose value agrees approximately with that of the heterozygous form. The partial dominance found in the F_1 generation persists here also. In subsequent generations, as with the leaf factor, the average value of the length of the vegetative period of the offspring approximates to that of the parent. In other words, the full sympodial and full monopodial forms will breed pure, but the intermediate forms will give rise to a series of forms with vegetative periods of varying lengths. There appears to be correlation between the habit and the presence of the red colouring matter of the sap; plants possessing the latter having a longer average vegetative period.

The behaviour of this character is, moreover, of great economic importance. It is essential that plants should be of the sympodial type if their cultivation in the United Provinces is to be a commercial success, for the monopodial types do not flower in time to give a crop before the winter sets in. At the same time, the majority of Indian cottons with a really valuable staple belong to the monopodial types. The chief hope of improvement of the cotton crop in the United Provinces, therefore, has been based on the isolation of pure sympodial forms with the staple of the monopodial type.

5. *Glands of the Leaf.*—The presence or absence of glands on the main veins of the leaf of the Types 4—9, and 11 affords a means of sub-dividing these types. Two pure conditions are recognisable—that in which the glands are absent (0), and that in which all the leaves of the main stem and monopodial branches bear three glands (3-1).* The other conditions, in which all the leaves bear one gland or, in general, those of

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* In this notation the first figure denotes the number of glands most commonly found on the leaves of the secondary monopodial branches, the second figure the number on the leaves of the main stem.

the main stem only bear three glands (1-3), appear to be impure. Such plants are produced by crossing the two pure forms (0) and (3-1). Type 2 appears to be an exception, being a pure form with leaf glands (1), while Types 3 and 10 have only been observed in the pure (3-1) condition. Type 1 has not been observed sufficiently to justify a definite statement.

The Alcoholic Ferment of Yeast-juice. Part VI.—*The Influence of Arsenates and Arsenites on the Fermentation of the Sugars by Yeast-juice.*

By Arthur Harden, F.R.S., and W. J. Young (Biochemical Laboratory, Lister Institute).

(Received January 17,—Read March 2, 1911.)

The rate of fermentation of a simple sugar by yeast-juice undergoes a temporary acceleration when a soluble phosphate is added.* The close chemical analogy which exists between phosphates and arsenates suggested the idea that this similarity might extend to the effect of arsenates on fermentation, and accordingly experiments were made to test the point. It was thus found that arsenates have a great effect on fermentation, but subsequent work has shown that it is of a totally different nature from that exerted by phosphates.

I. *Influence of Arsenate on the Rate of Fermentation.*

When a solution of sodium arsenate is added to a mixture of glucose, mannose or fructose with yeast-juice, which has attained a steady rate of fermentation, an immediate large increase in the rate of evolution of carbon dioxide is produced. The rate, as a rule, rises for a short period, attains a maximum, and then very gradually diminishes. A typical example is the following.

Experiment 1.—The experiments throughout were carried out at 25° in presence of toluene, the solutions being previously saturated with carbon dioxide. The standard solution of sodium arsenate employed was approximately 0·3 molar, and was prepared by dissolving 5·312 grammes of crystallised sodium arsenate in water, and making up to 100 c.c. Two separate mixtures were made, each containing 25 c.c. yeast-juice and 2·5 grm. of glucose, and were incubated until a steady rate of fermentation had been attained. There were then added (*a*) 25 c.c. of water and 2·5 grm. glucose; (*b*) 20 c.c. of water + 5 c.c. standard arsenate solution and 2·5 grm. glucose.

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* Harden and Young, 'Roy. Soc. Proc.,' B, 1906, vol. 77, p. 410.

The rates of evolution of carbon dioxide were then observed, the apparatus previously described being employed.

Time.	*a.*		*b.*	
	Total gas.	Average rate per 5 minutes during interval.	Total gas.	Average rate per 5 minutes during interval.
5	2·1	2·1	23·3	23·3
10	4·9	2·8	47·8	24·5
15	8	3·1	72·5	24·7
20	10·7	2·7	99·5	27
30	15·9	2·6	154·1	27·3
40	21·4	2·8	208·4	27·15
50	26·5	2·55	259·3	25·45
65	34·5	2·7	332·2	24·3
95	50·6	2·7	459·0	21·1

A striking feature of the effect of the addition of a phosphate to yeast-juice is that the marked acceleration only continues until an amount of carbon dioxide has been evolved which is chemically equivalent to the phosphate added. Moreover, at the close of this period of enhanced fermentation, the added phosphate is no longer present in a form precipitable by magnesium citrate mixture, but has become converted into a hexosephosphate. Neither of these phenomena occurs when an arsenate is substituted for the phosphate. The enhanced rate of fermentation continues long after an equivalent of carbon dioxide has been evolved, and no organic combination of arsenic is formed.

The first of these statements follows from the results of Experiment 1. Here the arsenate added was equivalent to 36 c.c. of carbon dioxide in the ratio $Na_2HAsO_4 : CO_2$, whilst the extra carbon dioxide evolved in the presence of the arsenate amounted to 408·4 c.c. in 95 minutes, and the rate at the end of that time was still more than seven times that of the juice without arsenate.

The sharp contrast between the actions of arsenate and phosphate is clearly shown when the effects of equivalent amounts of phosphate and arsenate on the same sample of yeast-juice are directly compared, as is done in the following experiment.

Experiment 2.—(1) 20 c.c. yeast-juice + 5 c.c. of 0·3 molar phosphate; (2) 20 c.c. yeast-juice + 5 c.c. of 0·3 molar arsenate; (3) 20 c.c. yeast-juice + 0·75 c.c. of 0·3 molar arsenate.

Excess of sugar was present in each case, and the solutions of phosphate and arsenate were each equivalent to 36 c.c. of carbon dioxide for 5 c.c. of the solution. The normal rate of fermentation of the juice was 1·8 c.c. per five minutes.

Time.	1.		2.		3.	
	Total.	Rate per 5 minutes.	Total.	Rate per 5 minutes.	Total.	Rate per 5 minutes.
5	7·1	7·1	11·4	11·4	21·7	21·7
10	19·8	12·7	26·5	15·1	46	24·3
15	36·1	16·3	43	16·5	71	25
20	43·8	7·7	59	16	95·3	24·3
25	45·7	1·9	75	16·3	119·8	24·5
30	47·5	1·8				

Calculating the extra amount of carbon dioxide evolved in each case beyond that due to the normal rate of the juice, it is found that, in presence of phosphate, this amounts to 36·7 c.c., and that the rate has fallen to that of the original juice. In presence of arsenate, although the extra amount of carbon dioxide is 66·3 c.c., the rate has remained unaltered at the high value of 16. The disparity between this extra amount of carbon dioxide and the equivalent of the arsenate added is still more strikingly illustrated when only a small amount of arsenate is added. This is shown in No. (3) above, to which only 0·75 c.c. of arsenate, equivalent to 5·4 c.c. of carbon dioxide, was added. In spite of this the extra amount of carbon dioxide evolved in 25 minutes was 120·8 c.c., and the rate at the end of that time was still 24·5 c.c. per 5 minutes.

In order to show that the whole of the arsenate is still present in its original state after the evolution of an equivalent amount of carbon dioxide, the following experiment was made:—

Experiment 3.—Four quantities of 25 c.c. of yeast-juice + 2·5 grm. of glucose were incubated until a steady rate had been attained.

To these were added:—

(1) 5 c.c. water.

(2) 5 c.c. of sodium arsenate solution, yielding 0·2211 grm. of $Mg_2As_2O_7$, and equivalent to 35 c.c. of carbon dioxide.

(3) 5 c.c. of sodium arsenate, as above, and 2·5 grm. of glucose.

(4) 5 c.c. of sodium phosphate equivalent to the arsenate added to (2), and yielding 0·1553 grm. of $Mg_2P_2O_7$.

The fermentations of 1 and 2 were then observed until the evolution of carbon dioxide from (2) was approximately equal to that from (1) + 35 c.c., and the two liquids were then boiled and filtered and the weight of precipitate yielded by magnesium citrate mixture ascertained.

Liquid (3) was allowed to ferment until a total of 713 c.c. had been evolved, and was then boiled and treated as above.

Finally (4), which was intended to demonstrate the different behaviour of phosphate, was allowed to ferment until the rate again became normal, and was then boiled and precipitated with magnesium citrate mixture.

The following table shows the total weight of precipitate produced in each case by magnesium citrate mixture:—

	Original juice.	Added.	Total before incubation.	Total after incubation.	Difference.
1	0·0678	0	0·0678	0·0678	0
2	0·0678	0·2211	0·2889	0·3007	+0·0118
3	0·0678	0·2211	0·2889	0·3513	+0·0624
4	0·0678	0·1553	0·2231	0·1025	−0·1206

These numbers clearly show that the whole of the arsenate remains precipitable by magnesium citrate mixture, even after prolonged fermentation, whereas the phosphate is converted into a non-precipitable form.

This fact is, however, not sufficient to exclude the possibility of the formation of an arsenic analogue of a hexosephosphate, since this might be precipitable by magnesium citrate mixture.

Experiment 4.—The precipitate obtained from another experiment conducted on similar lines to (2) was therefore carefully washed, dried at 100°, mixed with copper oxide, and submitted to combustion in a current of oxygen; 0·3621 grm. gave 0·0069 grm. of CO_2, corresponding with 0·052 per cent. of carbon. A magnesium hexose-arsenate would contain 15·3 per cent. of carbon, so that the result of this analysis shows that no appreciable amount of such a compound is present. The trace of carbon actually found was no doubt derived from occluded matter from the boiled yeast-juice in which the precipitate was produced. The arsenate therefore remains free throughout the fermentation.

2. *The Products of Fermentation in Presence of Arsenate.*

In order to ascertain whether the reaction which occurred in the presence of arsenate was the normal decomposition of sugar into alcohol and carbon dioxide, the ratio of these products was determined for an arsenate fermentation.

Experiment 5.—75 c.c. of yeast-juice were incubated with 8 grm. of glucose and 15 c.c. of 0·1 molar sodium bicarbonate solution in order to remove all free acid and free phosphate. In one portion of this the alcohol was estimated at once by distillation. To each of two other equal portions, 1 c.c. of arsenate solution was added, and both were incubated at 25° for 1·5 hours. During this period 185·3 c.c. of pure carbon dioxide, measured moist at 20·8° and 763·6 mm., were evolved, weighing 0·3303 grm. At the end of this time the total alcohol was estimated in one of the portions, and the alcohol produced in presence of arsenate thus ascertained by subtracting that originally present. The following were the results:—

	Before incubation.	After incubation.	Difference.	Ratio.
Alcohol	0·4898	0·7924	0·3026	0·92
Carbon dioxide	0	0·3303	0·3303	

The theoretical ratio is 1·04. The value obtained agrees well with the ratio previously found by similar methods for the normal fermentation of glucose by yeast-juice.

3. *Effect of the Concentration of Arsenate on the Maximum Rate of Fermentation Attainable.*

The rate attained increases with the concentration of arsenate until a certain limit is reached. A further increase beyond this point diminishes the rate of fermentation, so that just as in the case of phosphate there is a certain optimum concentration of arsenate, corresponding with which there is a maximum rate of fermentation.

In carrying out these estimations of rate of fermentation, a mixture of glucose with yeast-juice, 10 grm. to 100 c.c., was incubated until the rate of evolution of carbon dioxide had become constant, and all free phosphate had been converted into hexosephosphate. The standard sodium arsenate solution (0·3 molar), containing 10 grm. of glucose per 100 c.c. and saturated with carbon dioxide at 25°, was then added in the desired quantity. Since sodium arsenate solution after saturation with carbon dioxide contains an equilibrium mixture of the two arsenates and sodium bicarbonate, the amount of the latter present in the arsenate solution used was previously estimated by neutralising a known quantity with hydrochloric acid and measuring the carbon dioxide evolved. A solution of sodium bicarbonate, also containing 10 grm. of glucose per 100 c.c., was then made up which yielded the same amount of carbon dioxide when neutralised as the arsenate solution. The liquid in each flask was made up to the same volume with this sodium bicarbonate solution. The fermentation flasks containing the proper quantities of arsenate solution and bicarbonate were then incubated at 25° and the rates of fermentation observed. The effect of bicarbonate is to cause a slight increase in the rate of fermentation, owing to a diminution of the acidity of the juice (Buchner).

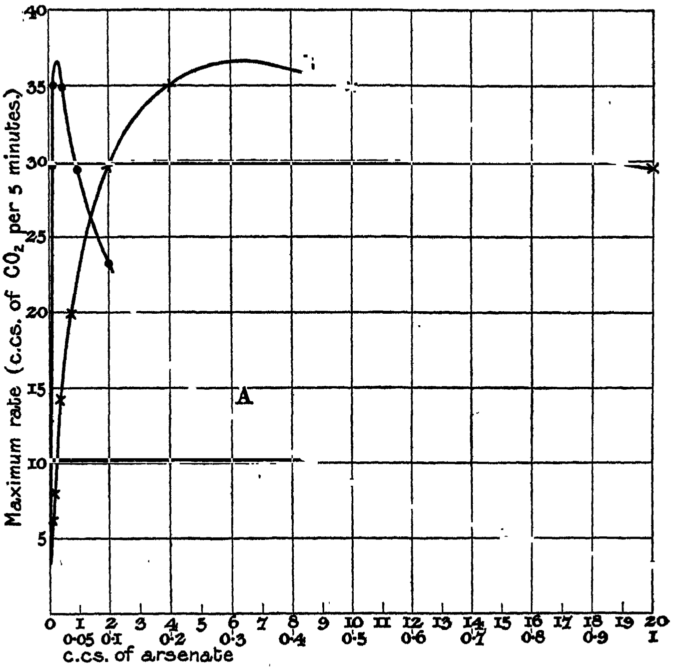
The following results, all obtained from a single sample of yeast-juice, are typical, and show the nature of the relation between concentration of arsenate and rate of fermentation.

When the concentration of arsenate is very low, the rate falls off very rapidly from the maximum, and the numbers obtained in these cases can only be regarded as approximate. This is the case in the present set of observations for amounts of arsenate less than 0·1 c.c.

Experiment 6.—20 c.c. of yeast-juice $+ x$ c.c. of 0·3 molar arsenate $+ (20-x)$ c.c. sodium bicarbonate solution. Glucose, 4 grm.

Arsenate solution per 40 c.c. mixture.	Molar concentration of arsenate in fermenting mixture.	Maximum rate of fermentation.
c.c.		c.c. per 5 minutes.
0	0	3·5
0·005	0·000037	6·3
0·01	0·000075	8·0
0·02	0·00015	14·2
0·04	0·0003	19·9
0·1	0·00075	29·7
0·2	0·0015	35·0
0·5	0·00375	34·9
1	0·0075	29·5
2	0·015	23·2
5	0·0375	14·5
10	0·075	8·7
15	0·1125	5·3
20	0·15	3·2

The same results are exhibited graphically in the accompanying curves, in which the rate is plotted against the cubic centimetres of arsenate present in the mixture. Curve B represents the earlier portion of A, plotted with a twenty-fold scale of abscissæ.



The striking feature of the curve is the rapid rise of rate with the concentration, followed by a fall, at first rapid and then gradual. The reason for this fall is discussed later on.

The smallest amount of arsenate recognisable by a change of rate of fermentation in this experiment corresponded with the presence of 0·11 mgrm. of arsenic in the fermenting liquid.

Both the optimum amount of arsenate and the maximum rate of fermentation produced by it vary with the sample of yeast-juice employed, and the following numbers illustrate this point :—

Experiment.	Volume.	Juice alone.	Maximum obtained.	Ratio.	Optimum molar concentration of arsenate.
		c.c. per 5 minutes.			
7	20	3·5	36·5	10·4	0·00225
8	20	1·3	14·2	10·9	0·01125
9	20	1·6	25	15·6	0·005625

4. *Effect of the Addition of Arsenate on the Total Fermentation produced by Yeast-juice.*

When arsenate is added to yeast-juice and sugar, the rate of fermentation more or less rapidly reaches the maximum corresponding with the concentration of arsenate employed. This rate is maintained for a varying time, according to the amount added, and then slowly falls off until ultimately fermentation ceases.

When the concentration of arsenate is comparatively high, fermentation ceases much sooner than in the absence of arsenate, and this appears to be due to some direct action on the fermenting complex, similar to that which was observed by Buchner as resulting from the addition of potassium arsenite, and which is discussed later on. When, on the other hand, a small concentration of arsenate is used a high rate is produced, fermentation continues for a longer period than in the absence of arsenate, and a greatly increased total fermentation results. The total amounts of carbon dioxide and alcohol produced therefore depend on the particular concentration of arsenate employed, and may be either higher or lower than that given by the juice in absence of arsenate.

Experiment 10 is a typical example of the phenomena observed when a moderate quantity of arsenate is used, while Experiment 11 exhibits the effect of variation of concentration of arsenate both on the rate and the total fermentation.

Experiment 10.—Parallel experiments were made with juice alone, and in the presence of arsenate, the solutions having the following compositions:—

1. 20 c.c. yeast-juice + 15 c.c. sodium bicarbonate + 5 c.c. arsenate.
2. 20 c.c. yeast-juice + 20 c.c. sodium bicarbonate.

Each solution contained 4 grm. of glucose.

Time.	1.		2.	
	Rate per 5 minutes.	Total evolved.	Rate per 5 minutes.	Total evolved.
h. m.	c.c.		c.c.	
15	9·5		2·1	
30	13		2·1	
45	14·2		1·9	
1	13·9	142·5	1·8	22·2
1 15	12·8		1·6	
1 30	11·9		1·5	
2	9·8	288	1·2	39·5
4	3·4	436	0·9	65·2
6	1·7	498	0·7	85
10	0·2	544	0·41	108
14	0	550	0·33	122
35			0	133

It will be seen that in the presence of arsenate (1) the maximum rate is attained in about 45 minutes, and amounts to 14·2 c.c. per 5 minutes; the total carbon dioxide evolved is 550 c.c., and fermentation ceases in about

12 to 14 hours. In the absence of arsenate (2), the maximum is attained at once and is 2·1, the total evolved is 133 c.c., and the duration of the fermentation is about 24 to 30 hours.

Experiment 11.—The totals evolved by equal volumes of the juice employed in the foregoing experiment were determined with various additions of arsenate, and are quoted along with the maximum rates and the approximate duration of fermentation.

No.	Arsenate added.	Maximum rate.	Total evolved.	Duration of fermentation.
	c.c.			hours.
1	0	2·8	133·3	24—30
2	0·5	25	1024	55—60
3	5	14·2	550	12—14
4	10	5·9	190·6	9—10
5	20	1·5	47·8	7

4 grm. of glucose were present in each case, and it is probable that fermentation in No. 2 came to a close on account of exhaustion of the sugar.

5. *The Fermentation of Mannose and Fructose by Yeast-juice in the Presence of Arsenate.*

The fermentation of mannose by yeast-juice is also accelerated by the addition of arsenate to about the same extent as that of glucose. In the case of fructose, however, a difference, similar in character to that previously noted in the presence of phosphate,* is also observed with arsenate, viz., the optimum concentration of arsenate is greater and the maximum rate attainable higher than in the case of glucose.

These facts are well brought out by the results of the following two experiments.

Experiment 12.—A series of different volumes of the standard arsenate solution were added to yeast-juice in presence of 1 grm. of the sugar in question, all the liquids being made up to the same volume, and the maximum rate attained in each case observed.

20 c.c. yeast-juice + x c.c. arsenate + $(26-x)$ c.c. of a solution containing 1 grm. of sugar.

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* Harden and Young, 'Roy. Soc. Proc.,' B, 1909, vol. 81, p. 336.

x.	Maximum rate for 5 minutes.		
	Glucose.	Mannose.	Fructose.
0	0·63	0·65	0·65
0·2	7·0		
0·5	9·1		11·7
1	9·8	8	14·4
2	9·7	6·6	16
4	4	4·5	14·8
8			8·4
16			2·4

Experiment 13.—A similar experiment was made with a mixture of 20 c.c. of yeast-juice and 2 grm. of the sugar, the total volume being 40 c.c.

Arsenate.	Maximum rate in 5 minutes.	
	Glucose.	Fructose.
c.c.		
0	1	1·2
0·2	7·2	
1	12·1	26·6
2	12·4	
3	13·4	
5	13·4	43·6
7		45·8
10		42·4
15	5·1	39

In this case the optimum concentration of arsenate in the presence of glucose is between 3 and 5 c.c., and the maximum rate is about 13·4, which is 13·4 times the normal rate. In the presence of fructose, on the other hand, the optimum concentration is about 7 c.c., and the maximum rate 45·8 c.c., which is 38·2 times the normal rate.

In Experiment 12 the juice evidently contains less fermenting complex, and there is not such a great disparity between the optima. The maximum rate for glucose is 9·8 c.c., for mannose, slightly less, 8 c.c., and for fructose, 16 c.c.

6. *Nature of the Effect of Arsenate on Fermentation by Yeast-juice.*

In considering the nature of the action of arsenate in accelerating the fermentation of sugars by yeast-juice two possibilities present themselves In the first place the arsenate may replace the phosphate in the main reaction :

$$(1)\quad 2C_6H_{12}O_6 + 2Na_2HPO_4 = 2CO_2 + 2C_2H_6O + 2H_2O + C_6H_{10}O_4(PO_4Na_2)_2.$$

The facts that much more than an equivalent of carbon dioxide is evolved at the accelerated rate and that the arsenate is found in the free state at every stage of the fermentation render it necessary to suppose that any arsenic compound which is formed is of a very unstable nature, and only exists at any moment in a very low concentration.

On the other hand, it is also possible that the arsenate accelerates the fermentation by increasing the rate at which free phosphate is produced from the hexosephosphate. This substance is hydrolysed by the enzyme hexosephosphatase according to the equation:

$$(2)\quad C_6H_{10}O_4(PO_4Na_2)_2 + 2H_2O = C_6H_{12}O_6 + 2Na_2HPO_4,$$

and it is the rate of this reaction which is the controlling factor of the rate of fermentation of sugar by yeast-juice in the absence of added phosphate.*

Acceleration of this change would therefore result in a constant increased supply of phosphate, and would enable reaction (1) to proceed at a correspondingly greater rate. In other words, the effect of the arsenate would be to produce a condition similar to that existing when free phosphate is added gradually to yeast-juice so that its concentration is maintained at the optimum, and the reaction proceeds at its maximum velocity as long as this condition is maintained.†

It also follows from the fact that the rate of fermentation is dependent upon the rate of production of free phosphate that the acceleration produced by the addition of arsenate cannot be due to an increase in the activity of the zymase alone. In a mixture of fresh yeast-juice and sugar this enzyme is not producing its maximum effect, as is shown by the fact that the addition of phosphate causes a large increase in the rate of fermentation. In other words there is an excess of enzyme already present in the mixture. An increase in the activity of the zymase, therefore, without an increase in the supply of phosphate, could not be expected to cause any acceleration in the rate of fermentation. It is possible, however, that the activity both of the hexosephosphatase and of the zymase might be simultaneously increased by the addition of arsenate. In this case it would be possible to obtain a higher maximum by the addition of arsenate in presence of the right amount of phosphate, than could be obtained by adding phosphate alone.

1. *Proof that Arsenate cannot replace Phosphate in the Fermentation of Sugars by Yeast-juice.*—The first of these alternative explanations, according to which arsenate is supposed to take the place of phosphate and to react with the sugar to form an unstable hexose-arsenate, can be investigated in

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* Harden and Young, 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 299.

† Harden and Young, 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 309.

the following way. A mixture of ferment and co-ferment can be prepared as previously described, which is free from phosphate and hexosephosphate,* and will not ferment sugar without the addition of phosphate. If now arsenate can replace phosphate, such a mixture should be rendered active by the addition of arsenate as well as by that of phosphate.

In order to realise these conditions zymin was freed from phosphates and co-ferment by repeated washing with water, until it was incapable of fermenting sugar. To this was added a solution of co-ferment, free from mineral phosphates and hexosephosphate, as previously described. This mixture was almost incapable of fermenting sugar, whilst the addition of phosphate, either in the form of mineral phosphate or hexosephosphate, readily caused fermentation. When, however, arsenate was substituted for the phosphate no fermentation ensued. This was not due to any inhibiting action of the arsenate, as in the presence of phosphate the same quantity of arsenate did not interfere with the fermentation.

Exp.	Zymin suspension.	Co-ferment solution.	Water.	Fructose.	Phosphate, 0·3 molar.	Hexose-phosphate.	Arsenate, 0·3 molar.	Gas evolved.			
	c.c.	c.c.	c.c.	grm.	c.c.	c.c.	c.c.	c.c.		hrs.	mins.
16. 1	10	20	1	2	0	0	0	7	in	24	
2	10	20	0	2	1·0	0	0	67	„	24	
3	10	20	0·75	2	0	0	0·25	0·2	„	2	
4	10	20	0	2	0	0	1	2·7	„	24	
5	10	20	0·25	2	0·5	0	0·25	51·6	„	24	
17. 1	10	20	6·3	0·7	0	0	0	1·5	„	19	
2	10	20	0	0·7	0	6·3	0	132·7	„	19	
3	10	20	6·3	0·7	0	0	0·5	1	„	19	
4	10	20	0	0·7	0	6·3	0·5	133·5	„	19	
18. 1	10	15	0	0·5	0	0	0·2	2·0	„	20	
2	10	15	8·8	0·5	0	8·8	0	25·8	„	1	50
3	10	15	0	0·5	0	8·8	0·2	47·8	„	1	50

The hexosephosphate solution used was 0·21 molar for Experiment 17 and 0·14 molar in Experiment 18.

It will be seen that in no case was arsenate able to take the place of phosphate, and it must therefore be concluded that the action of arsenate is fundamentally different from that of phosphate.

Incidentally the foregoing experiments afford further evidence that phosphate is indispensable for the alcoholic fermentation of sugar. Comparing Nos. 1 and 2, Experiment 17, it is seen that in the almost complete absence of available phosphate only 1·5 c.c. of carbon dioxide were evolved, whereas in the presence of hexosephosphate 132·7 c.c. were obtained. The ratio of the totals produced in presence and in absence of phosphate is thus 132·7/1·5 = 88, a much greater value than had previously been observed.*

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* 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 322.

2. *Acceleration of the Action of Hexosephosphatase by Arsenate.*—The direct replacement of phosphate by arsenate being excluded, the alternative explanation, that the arsenate accelerates the action of the hexosephosphatase, remains to be considered. This question can readily be investigated experimentally, and the result of such an examination is to show that the arsenate does in fact produce a very marked effect of this kind, and that it is to this acceleration that the enhanced rate of fermentation in the presence of arsenate is due. This has been proved separately for yeast-juice and zymin.

In the three experiments carried out with yeast-juice, a comparison was made between the rates of fermentation in mixtures of yeast-juice with (*a*) hexosephosphate and arsenate; (*b*) water and arsenate; (*c*) hexosephosphate alone.

In experiments such as these, done in the absence of added sugar, the rate of fermentation is controlled by the production of sugar (1) from the hexosephosphate; and (2) from the glycogen and dextrins of the juice. Hence, by subtracting the rate of auto-fermentation in presence of arsenate (*b*) from the rate of fermentation of hexosephosphate also in presence of arsenate (*a*) a difference is obtained which represents the share of this enhanced rate which is due to the hydrolysis of the hexosephosphate. This is given by $a-b$, and a comparison of this with the rate of fermentation of hexosephosphate in absence of arsenate shows whether the effect of the arsenate has been to increase the rate of hydrolysis of the hexosephosphate. (In order to obtain the true rate of evolution due solely to the hexosephosphate in absence of arsenate the rate of auto-fermentation of the juice should be subtracted from that observed in presence of hexosephosphate (*c*). As, however, it is not certain what effect the presence of hexosephosphate may have upon the enzymic hydrolysis of glycogen, etc., this has not been done in the present instance. The effect of making this subtraction would be considerably to increase the cogency of the argument.)

In order as far as possible to diminish the auto-fermentation, the yeast-juice employed in Experiments 19 and 20 was incubated for some time before the addition of the hexosephosphate and water, and the yeast employed for the preparation of the juice for Experiment 21 was treated for the removal of glycogen by the method described by Buchner and Mitscherlich, but still yielded a juice which gave a considerable amount of auto-fermentation.

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* 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 324.

Experiments 19, 20, 21.—In each case 25 c.c. of yeast-juice + 0·5 c.c. of 0·3 molar arsenate + 20 c.c. of water or a normal solution of sodium hexosephosphate (containing 8·5 grm. of the acid per 100 c.c.) were employed, the liquids being made up to 45·5 c.c.

No. of experiment.	Time after addition of arsenate, in minutes.	Total c.c. evolved in the time *t*.			
		a. Arsenate + hexosephosphate.	*b.* Arsenate + water.	*a*—*b*.	*c.* Hexosephosphate + water.
19	15	40·5	26·2	14·3	6·3
	30	77·7	50·5	27·2	12·1
	75	147·9	90·6	57·3	22·3
	205	311·7	165·9	145·8	55·4

No. of experiment.	Time after addition of arsenate.	Rate in c.c. evolved in preceding 5 minutes.			
		a.	*b.*	*a*—*b*.	*c.*
	h. m.				
20	5	2·9	0·5	2·4	1·4
	10	3·7	0·6	3·1	0·9
	15	3·7	0·4	3·3	0·8
	20	3·7	0·5	3·2	0·9
	25	3·9	0·3	3·6	0·7
	30	3·5	0·4	3·1	0·7
	Total...	21·4	2·7	18·7	5·4
21	5	9·1	3·0	6·1	3·1
	10	8·6	2·3	6·3	2·9
	15	7·3	1·9	5·4	0·9
	15 20	6·8	1·2	5·6	1·2
	35	6·4	1·3	5·1	1·3
	40	6·9	1·7	5·2	1·6
	45	6·1	1·3	4·8	0·9
	50	5·6	1·0	4·6	0·8
	Total...	56·8	13·7	43·1	12·7

It will be seen that in every case the fermentation in presence of hexosephosphate and arsenate, less the auto-fermentation in presence of arsenate (*a*—*b*) is considerably greater (two to four times) than the rate of fermentation in absence of arsenate (*c*).

As this increased fermentation is carried on at the expense of the hexosephosphate, it follows that the hexosephosphate is being more rapidly decomposed than in the absence of arsenate, and that therefore free phosphate, which is also a product of the hydrolysis of this compound, must also be liberated in greater quantity. Since, moreover, hexosephosphate yields two equivalents of phosphate for one of sugar the phosphate must accumulate, and accordingly the rate of decomposition of the hexosephosphate can also be gauged by the rate of accumulation of free phosphate.

Experiment 22.—This has been ascertained by estimating the amount of free phosphate in the boiled and filtered solutions from Experiment 19. The following are the results, expressed in grams of $Mg_2P_2O_7$, the equivalent of the arsenate present having been subtracted in each case:—

(*a*)	Hexosephosphate + arsenate................	0·6136
(*b*)	Auto-fermentation + arsenate	0·1144
(*c*)	Hexosephosphate without arsenate	0·0707

Hence, in presence of arsenate, 0·6136 − 0·0707 = 0·5429 grm. has been liberated more than in its absence, and of this amount 0·5429 − 0·1144 = 0·4285 grm. has been formed from the hexosephosphate which was added.

This result is therefore in entire agreement with the observations of the rates of fermentation.

Experiments with zymin confirm the conclusion that the arsenate accelerates the action of the hexosephosphatase in an equally striking manner. Zymin washed free from soluble phosphates and co-ferment was used, and a solution of co-ferment also almost free from available phosphate was added, together with an excess of fructose. This mixture was almost incapable of producing fermentation, owing to lack of phosphate, and as a matter of fact gave a total of only 1·5 c.c. of carbon dioxide, whereas when phosphate was added as much as 132 c.c. were involved. Solutions of hexosephosphate were added to two equal volumes of this mixture, and to one of these arsenate was also added. The sole source of phosphate in these two liquids was the hexosephosphate, and the rate of fermentation would therefore be controlled by the rate of production of phosphate from this. Any increase of fermentation in the presence of arsenate must therefore be due to the accelerating action of this salt on the hexosephosphatase.

Experiment 23.—The zymin and co-ferment solution were prepared in the same manner as those used in Experiment 17.

1. 10 c.c. zymin suspension + 15 c.c. co-ferment solution + 0·5 grm. fructose + 8·8 c.c. of 0·14 molar hexosephosphate + 0·2 c.c. arsenate.

2. The same mixture but containing 0·2 c.c. of water instead of arsenate.

Here the rate of fermentation, and consequently of hydrolysis of hexosephosphate, in presence of arsenate is about double that in its absence.

Time after addition of arsenate, in minutes.	C.c. evolved in preceding 5 minutes.	
	1.	2.
5	2·8	1·4
10	2·7	1·4
15	2·5	1·2
20	2·3	1·0
25	2·3	—
65	2·4	1·3
90	2·2	1·3
115	1·84	1·04
Total in 115..............	47·8	25·8

Whether the arsenate also accelerates the action of the zymase has not been decided with certainty. If this were so, the rate of fermentation attainable by the proper mixture of arsenate and phosphate would be greater than that given by phosphate alone. Since, however, the rate of fermentation in presence of phosphate falls off with diminishing concentration of this salt extremely rapidly and according to a law which has not yet been ascertained, it is impossible at present to institute an accurate comparison between the velocity constants of the two reactions. It may, however, be noted that cases have been observed in which the evolution of gas in five minutes was greater in presence of a certain quantity of arsenate than could be obtained with phosphate alone.

7. *Effect of Arsenate on the Auto-fermentation of Yeast-juice.*

When arsenate is added to yeast-juice which is undergoing auto-fermentation, a very considerable rise is produced in the rate of evolution of carbon dioxide; the rate attained is, however, much lower than that produced in presence of excess of glucose. The extent of this enhanced auto-fermentation is shown by the following (Nos. 1 and 2), whilst the effect of the same quantity of arsenate in presence of excess of glucose is given in columns 3 and 4.

Experiment 14.—Four solutions were incubated, all being made to the same volume of 30·5 c.c.

1. 20 c.c. yeast-juice + 10·5 c.c. water + 0 c.c. arsenate.
2. „ „ + 10 „ + 0·5 „
3. „ „ + 9·3 „ + 0 „ + 2 grm. glucose.
4. „ „ + 8·8 „ + 0·5 „ + 2 „

The following rates were observed:—

	No. 1.	No. 2.	No. 3.	No. 4.
Initial rate per 5 minutes	1·5	8·5	2·5	36·5
Total in 1 hour 40 minutes	27·2	81·2		

The rate of auto-fermentation of yeast-juice is not, like the rate of fermentation of sugar, controlled by the rate at which phosphate is produced from the hexosephosphate, but by that at which sugar is formed by the hydrolysis of glycogen and dextrins, as is shown by the well known fact, confirmed in the foregoing experiment, that the rate of auto-fermentation is usually considerably less than that of the fermentation of sugar.

Consequently, any increase in the rate of auto-fermentation must be primarily due to an increased supply of sugar. This may be derived from two sources. A portion is supplied, as has been shown (Experiments 19–23), by the increased rate of hydrolysis of the hexosephosphate originally present in the juice. This continues until the whole of the sugar obtainable from this source has been converted into carbon dioxide and alcohol and has the effect of temporarily increasing the rate of fermentation. This acceleration is independent of the presence or absence of glycogen, and hence the effect of arsenate on the juice in the absence of added glycogen gives a superior limit to the extent of its influence.

After this point has been reached, the sugar formed by the hydrolysis of complex carbohydrates becomes the sole source of carbon dioxide. As fermentation then proceeds this supply also gradually becomes exhausted and the rate falls off slowly. In presence of added glycogen, on the other hand, the rate falls off more slowly.

In order, therefore, to ascertain whether the rate of hydrolysis of glycogen is increased by the addition of arsenate, it is necessary to determine whether the effect of adding glycogen and arsenate together to the juice is greater than the sum of the effects of adding glycogen and arsenate to separate portions of the juice.

This is done in the following experiment:—

Experiment 15.—Five quantities of 20 c.c. of yeast-juice were mixed with the following solutions and incubated for 1 hour 45 minutes, the total volume in each case being 30·5 c.c.—

1. 2·5 grm. glucose in 10·5 c.c. solution.
2. 10·5 c.c. water.
3. 10 c.c. water + 0·5 c.c. arsenate.
4. 0·5 grm. yeast-glycogen in 10·5 c.c. solution.
5. 0·5 grm. yeast-glycogen in 10 c.c. solution + 0·5 c.c. arsenate.

	1.	2.	3.	4.	5.
Initial rate (c.c. per 5 minutes)	2·5	1·5	8·5	1·9	10·0
Rate after 1 hour	—	1·4	2·9	1·6	7·2
Rate after 1 hour 40 minutes	—	1·1	1·85	1·35	4·45
Total evolved in 1 hour 45 minutes	—	27·0	81·2	32·4	141·2

It is to be noted, in the first place, that the rate in presence of glycogen (1·9) is less than in presence of sugar (2·5), so that the rate of fermentation in No. 4 is actually controlled by the rate of production of sugar.

The effect of adding glycogen to the juice increases the total by (4)-(2)	32·4 − 27	=	5·4 c.c.
The effect of adding the arsenate to the juice alone increases the total by (3)-(2)	81·2 − 27	=	54·2 „
Sum of the separate effects of glycogen and arsenate	54·2 + 5·4	=	59·6 „
Effect of adding glycogen and arsenate together (5)-(2)	141·2 − 27	=	114·2 „

Hence at least 114·2 − 59·6 = 54·6 c.c. more carbon dioxide have been derived from glycogen in the presence of arsenate than in its absence. Comparing the rates per five minutes given in columns 3 and 5, showing the effect of arsenate on the fermentation in absence and in presence of added glycogen, it is to be noticed that the initial rates are not greatly different (8·5 and 10). This is due to the fact that at the commencement of the auto-fermentation there is an ample supply of glycogen. This, however, soon begins to fail, and consequently the rate falls off more rapidly than in the presence of added glycogen, the relative rates after 1 hour being 2·9 and 7·2, and after 1 hour 40 minutes, 1·85 and 4·45. For this reason the comparison is best made between the totals evolved after some time and not between the initial rates.

Further evidence in support of the conclusion that the hydrolysis of glycogen is accelerated by the addition of arsenate is afforded by the effect of the addition of glycogen to Solutions 2 and 3 in the foregoing experiment, which had been allowed to auto-ferment in absence and presence of arsenate respectively.

	No. 2.	No. 3.
5 minutes' rate before addition of glycogen	1·1	1·85
„ „ after	1·4	4·4

Even this enhanced rate of glycogen hydrolysis is not nearly rapid enough to supply sufficient sugar for the fermenting complex in presence of arsenate. The maximum rate obtained with glycogen was 1.0, whereas the addition of 0·5 c.c. of arsenate to Solution 1 containing excess of glucose produced a rate of 36·5 c.c. in five minutes.

It follows from the foregoing that arsenate has the power of greatly accelerating the action of the diastatic enzyme in yeast-juice.

8. *Action of Arsenite on the Fermentation of Sugar by Yeast-Juice.*

Buchner* made a number of observations on the effect of arsenite on the fermentation of sugars by yeast-juice. The presence of 2 per cent. of arsenious oxide, in the form of the potassium salt, was found in some cases completely to inhibit the fermentation of glucose, but not of saccharose, its effect on fructose being of an intermediate character, whilst, in other cases, the same concentration caused a marked increase in the total fermentation of all three sugars.

A powerful inhibitory effect was also produced by this concentration of arsenite, even on the fermentation of saccharose by (*a*) juice which had been prepared from yeast which had been kept at 8° for three days, (*b*) juice which had been dialysed against water for 16 hours, (*c*) juice which had been evaporated at 35°, (*d*) juice diluted with one volume of water.

It was further observed, in many cases, that the presence of arsenite caused a considerable increase in the fermentation during the first 16 hours, even when the total fermentation was less than in the absence of arsenite. It was found that this inhibitory action was not due to the conversion of the arsenite into arsenate; and, further, that it was lessened by the addition of heated yeast-juice or blood serum, either heated or unheated. The inhibitory action, moreover, was greater when the arsenite was incubated with the juice in the absence of added sugar. Buchner advanced no explanation of the preliminary acceleration caused by arsenite, and regarded the inhibitory effect as due to some change in the colloidal state of the enzyme, against which the presence of other colloidal substances partially protected it. It was, according to this, the diminution in the amount of these protecting colloids which made the samples of juice submitted by him to various operations so much more susceptible to the action of arsenite.

A careful experimental comparison of the effects of arsenite and arsenate on fermentation by yeast-juice shows that these two actions are precisely similar in kind, but that the acceleration produced by arsenite is markedly less than that caused by an equivalent concentration of arsenate. This similarity of the action of arsenite to that of arsenate is shown in the experiments summarised below.

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* 'Die Zymasegärung,' pp. 184—205.

(1) Arsenite produces an acceleration which continues long after an extra amount of carbon dioxide equivalent to the arsenite added has been evolved.

Experiment 24.—Yeast-juice containing 10 grm. of glucose per 100 c.c. was incubated until a steady rate of fermentation had been attained. To 20 c.c. of this were added 5 c.c. of a 0·3 molar solution of sodium arsenite, equivalent to 33·6 c.c. of carbon dioxide at N.T.P., and the rate of fermentation observed.

Time after addition, in minutes.	Rate in c.c. of carbon dioxide evolved in preceding 5 minutes.	Total.	Time after addition, in minutes.	Rate in c.c. of carbon dioxide evolved in preceding 5 minutes.	Total.
0	1·7		25	6·2	32·8
5	7·0	7·0	30	5·9	38·7
10	7·1	14·1	45	5·6	56·5
15	6·2	20·3	60	5·6	73·2
20	6·3	26·6	85	3·3	89·7

The total evolved in 85 minutes is thus 89·7 c.c., whereas in absence of arsenite it would have been 17 × 1·7 = 28·9, and hence the extra carbon dioxide is 89·7 − 28·9 = 60·8, which is largely in excess of the equivalent, 33·6.

(2) The acceleration produced by arsenite is much less than that obtained with an equivalent amount of arsenate.

Experiment 25.—20 c.c. of yeast-juice, containing 2 grm. of glucose, gave a rate of 1·7 c.c. in five minutes. The addition of 1 c.c. of a 0·3 molar solution of arsenite raised the rate to 3·1 c.c. in five minutes, whilst the addition of the same volume of 0·3 molar arsenate to the original juice gave a rate of 27·5 c.c.

(3) The rate attained increases with the concentration of the arsenite until a maximum is reached, so that, as in the case of arsenate, there is an optimum concentration of arsenite.

Experiment 26.—Varying quantities of a 0·3 molar solution of sodium arsenite were added to 20 c.c. of yeast-juice, the total volume being maintained at 40 c.c., and containing 10 per cent. glucose.

(4) The maximum rate attainable and the optimum concentration of arsenite are both greater in the case of fructose than in that of glucose.

C.c. arsenite added.	Rate in c.c. of carbon dioxide in 5 minutes.
0	2·1
1	3·4
3	6·4
5	6·9
13	4·5
20	0·8

Experiment 27.—The numbers in the following table refer to 20 c.c. of yeast-juice and 1 grm. of the sugar in 35 c.c. total volume.

C.c. molar arsenite added.	Rate in c.c. per 5 minutes.	
	Glucose.	Fructose.
0	0·34	0·5
2·5	1·17	1·45
5	2·7	3·5
10	2·7	7·96
15	1·48	9·8

It will be noted that the optimum concentration was not attained for fructose, whilst for glucose it was between 5 and 10 c.c.

(5) When a mixture of ferment, co-ferment, and sugar is rendered so free from phosphate that no fermentation will take place without the addition of phosphate, this added phosphate cannot be replaced by arsenite. Arsenite therefore cannot exercise the same function in alcoholic fermentation as phosphate.

Experiment 28.—The following experiment was carried out in a similar manner to Experiment 17 dealing with arsenate.

To equal volumes of a mixture of 10 c.c. of washed zymin suspension + 20 c.c. co-ferment solution free from phosphate + 0·22 grm. of fructose were added the following:—

		C.c. CO_2 in 18 hours.
1.	6·5 c.c. water	1·5
2.	6 „ + 0·5 c.c. phosphate	38
3.	6 „ + 0·5 „ arsenite	1·4
4.	6 c.c. normal hexosephosphate + 0·5 c.c. arsenite	33

The result of (4) shows that the amount of arsenite present was not sufficient to inhibit the action of the enzyme, whilst (3) shows that arsenite alone produced no increase in fermentation.

(6) Arsenite accelerates the rate of hydrolysis of glycogen, and hence the auto-fermentation of yeast-juice.

Experiment 29.—The following solutions were incubated and the rates of fermentation observed :—

1. 20 c.c. of yeast-juice from well-washed yeast + 14 c.c. water.
2. 20 c.c. of the same juice + 9 c.c. water + 5 c.c. of 0·3 molar arsenite.

Experiment 30.—In this case 25 c.c. of yeast-juice were used along with (1) 15 c.c. of water; (2) 10 c.c. water and 5 c.c. of 0·3 molar arsenite.

Experiment.	Time, in minutes.	Auto. + water.		Auto. + arsenite.	
		Rate in 5 minutes.	Total.	Rate in 5 minutes.	Total.
29	5	0·6	0·6	2·6	2·6
	10	0·5	1·1	2·2	4·8
	15	0·8	1·9	2·2	7·0
	20	0·5	2·4	2·4	9·4
	60	0·55	6·8	2·6	26·7
30	5	0·9	0·9	4·5	4·5
	10	1·0	1·9	4·0	8·5
	15	1·0	2·9	—	—
	20	1·4	4·3	3·8	16·1
	60	1·3	9·8	4·3	33·5

That this increase is, at all events in part, due to an acceleration of the rate of hydrolysis of glycogen is shown in precisely the same manner as for arsenate (Experiment 15). The results obtained are as follows :—

Experiment 31.—Five quantities of 25 c.c. of yeast-juice were incubated with the following solutions and the fermentations observed :—

1. 0·5 grm. glucose in 15 c.c. solution.
2. 15 c.c. water.
3. 10 c.c. water + 5 c.c. of 0·3 molar arsenite.
4. 0·5 grm. glycogen in 15 c.c. solution.
5. 0·5 grm. glycogen in 10 c.c. solution + 5 c.c. arsenite.

	1.	2.	3.	4.	5.
Initial rate (c.c. per 5 minutes) ...	3·0	1·3	4·0	1·6	4·2
Total evolved in 5 hours	—	67·8	118·3	73·7	152·1

Here, as in the experiment with arsenate, the rate in presence of glycogen (1·6) is less than that in presence of glucose (3·0), so that the rate of the fermentation in (4) is actually controlled by the rate of production of sugar.

The effect of adding glycogen to the juice is to increase the total by	73·7 − 67·8 = 5·9
The effect of adding the arsenite alone to the juice is to increase the total by	118·3 − 67·8 = 50·5
Sum of separate effects of glycogen and arsenite ...	50·5 + 5·9 = 56·4
Effect of adding glycogen and arsenite together is	152·1 − 67·8 = 84·3

Hence at least 84·3 − 56·4 = 27·9 c.c. more carbon dioxide have been derived from the glycogen in the presence of arsenite than in its absence in the same time.

(7) Arsenite produces its effect by accelerating the action of the hexosephosphatase.

Experiment 32.—The experiments on this point were carried out exactly as described under arsenate (Experiments 19, 20, 21), but, in addition, the auto-fermentation of the juice (*d*) was observed—

t.	Total c.c. evolved in time *t*.					
	a.	*b*.	*c*.	*d*.	*a*−*b*.	*c*−*d*.
5	4·1	2·6	1·0	0·6	1·5	0·4
10	7·7	4·8	2·2	1·1	2·9	1·1
15	11·2	7·0	3·4	1·9	4·2	1·5
20	14·8	9·4	4·6	2·4	5·4	2·2
25	17·9	11·7	5·6	2·8	6·2	2·8
55	35·7	24·2	10·7	6·2	11·5	4·5
60	38·7	26·7	12·3	6·8	12·0	5·5
65	41·7	28·8	13·2	7·3	12·9	5·9

As already explained, the original fermentation due to hexosephosphate alone is given by *c*−*d*, and the enhanced fermentation due to hexosephosphate by *a*−*b*. The effect of arsenite on the hexosephosphatase is very much less than that of arsenate, as was to be expected from the relative effects of the two on the fermentation of sugar, and is only clearly visible when the original auto-fermentation is subtracted from the fermentation in presence of hexosephosphate. The result in this case therefore depends on the assumption that the presence of the hexosephosphate and the products of its hydrolysis does not seriously affect the rate of hydrolysis of the glycogen.

Experiment 33.—An experiment was also carried out with washed zymin in precisely the same way as described for arsenate (Experiment 23), hexosephosphate being made the sole source of phosphate.

10 c.c. of washed zymin suspension + 15 c.c. of co-ferment solution free

from phosphate + 0·5 grm. of fructose + 9 c.c. of hexosephosphate solution + 1 c.c. of water, on addition of—

(1) 1 c.c. water gave 9·5 c.c. of carbon dioxide in 25 minutes.

(2) 1 c.c. of 0·3 molar arsenite solution gave 11·1 c.c. in 25 minutes.

In this case the rate was only increased about 20 per cent., whereas the addition of arsenate doubled the rate.

9. *Inhibitory Action of Arsenate and Arsenite.*

Both arsenate and arsenite may exert an inhibitory action on alcoholic fermentation, and this may manifest itself in two ways. If a sufficient excess be added, no fermentation at all occurs; in presence of a smaller amount than is necessary to produce this total inhibition, the effect is manifested by the low rate and shortened duration of fermentation. An experiment showing this behaviour of arsenate in a typical manner has already been quoted (No. 11). Even when the rate of fermentation is considerably increased by the presence of an amount of arsenate in excess of the optimum amount, it frequently happens that this rate falls very rapidly and that all fermentation ceases in an hour or two. The exact conditions under which this occurs have, however, not been ascertained. It is as yet uncertain whether this inhibitory action is due, as suggested by Buchner, to changes in the colloidal state of the fermenting complex or to the formation of inactive combinations of the complex with the arsenate and arsenite, in a similar manner to that suggested by the authors in the case of inhibition produced by excess of phosphate.* In all probability the phenomenon is a complex one in which several factors participate.

The total inhibition produced by arsenite which was observed by Buchner (p. 469) with juice which had been submitted to dialysis, evaporation, etc., may not improbably be ascribed to a loss of fermenting complex during these operations, so that the amount of arsenite added was greatly in excess of the optimum. It is, however, quite possible that the proteins accompanying the ferment present in the juice do exert a protective influence, as suggested by Buchner, and that a diminution in their amount may also play some part in the phenomenon.

10. *Experiments with Various Salts.*

No other salts capable of bringing about results in any way comparable with those produced by arsenates and arsenites have been found. Experiments with the sulphate, orthovanadate, antimonate, tungstate, chromate, stannate, phosphite, and citrate of sodium, and with tartar emetic and zinc sulphate, were all negative.

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* 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 299.

Summary.

1. When a suitable amount of arsenate is added to a fermenting mixture of yeast-juice and a sugar, it causes a large acceleration in the rate of production of carbon dioxide and alcohol. This enhanced rate differs from that produced by phosphate, inasmuch as it continues long after a chemical equivalent of carbon dioxide has been evolved. The arsenate, moreover, is found in the free state throughout the fermentation.

2. The rate attained increases rapidly with addition of arsenate, until an optimum concentration is reached, after which it decreases, at first rapidly, and then more slowly.

3. The total fermentation produced depends on the particular concentration of arsenate employed, and may be either higher or lower than that given in the absence of arsenate. As the high rate produced by a suitable quantity of arsenate persists for a long time, very considerable increases in the total fermentation may be observed.

4. Glucose and mannose are similarly affected by yeast-juice in presence of arsenate, whereas fructose is much more rapidly fermented than these two sugars, and the optimum concentration of arsenate in its presence is greater.

5. The increased rate of fermentation of sugars in presence of arsenate is due to an acceleration of the rate of action of the hexosephosphatase of the juice, whereby an increased supply of phosphate is afforded. The action is therefore essentially different from that of phosphate, and it has been found that arsenate cannot replace phosphate in the fundamental reaction of alcoholic fermentation.

6. Arsenate also causes a considerable increase in the rate of the auto-fermentation of yeast-juice, and in the rate of the fermentation of glycogen. This is mainly due to an acceleration of the rate of action of the diastatic enzyme of yeast-juice (glycogenase).

7. The action of arsenites is similar to that of arsenates, but is much less marked.

8. Both arsenate and arsenite cause total inhibition of the fermentation when they are present in a high concentration, but the nature of this effect has not been ascertained.

Gametogenesis of the Gall-Fly, Neuroterus lenticularis.—Part II.

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(Communicated by Prof. W. Bateson, F.R.S. Received January 25,—
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[Plate 17.]

Introduction.

In the first part of this paper* I described the life-history, spermatogenesis, maturation of the fertilised egg, fertilisation and segmentation divisions of the summer generation of the gall-fly *Neuroterus lenticularis* (*Spathegaster baccarum*), and gave some observations on the maturation of the eggs of the spring generation, and on the somatic mitoses in males and females. These latter observations were admittedly incomplete owing to lack of material, and this second part of the paper attempts to complete the account of what was left uncertain in the first communication. Before proceeding to describe my fresh observations it may be well shortly to summarise the results arrived at in the first part.

The spring generation of *N. lenticularis* consists of females which lay parthenogenetic eggs; those laid by some females develop into males, those laid by others develop into sexual females, both of which hatch in June. The eggs of the summer females undergo a double maturation division, leaving 10 chromosomes in the nucleus of the mature egg. The spermatogonia of the males in the same generation have 10 chromosomes; the first spermatocyte division is suppressed; in the second spermatocyte division the chromosomes divide, 10 entering each spermatid. A small staining extra-nuclear body found in the spermatocytes does not divide, and is included in only half of the spermatids. In fertilisation, the egg and sperm nuclei each contain 10 chromosomes, and the segmentation divisions of the fertilised egg show 20. For a complete study of the maturation and segmentation of the parthenogenetic spring eggs my material was insufficient, but it suggested that the eggs laid by some females underwent maturation and showed 10 chromosomes in the segmentation divisions, and that others had no polar divisions and showed 20. Since somatic and oogonial mitoses of the summer female have 20 chromosomes, but the spermatogonial mitoses and those of the developing nervous system in the male only 10, it was suggested that the eggs which undergo maturation produce males, the others females. An attempt was made, in conclusion, to connect these phenomena with what is known of sex-determination in other animals.

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* 'Roy. Soc. Proc.,' B, vol. 82, p. 88.

The material on which the observations here recorded were made was collected in March and April, 1910; the methods adopted were essentially those described in Part I, the eggs laid by each female being fixed and preserved separately at intervals from one to nine hours or more after being laid.

In addition to Petrunkewitsch's fluid another formula of alcoholic sublimate was tried (50 per cent. alcohol with sublimate to saturation + 6 per cent. acetic acid), but the results obtained were inferior to those yielded by Petrunkewitsch's mixture. Additional experiments were made to test the conclusion that the eggs laid by any fly are all male-producing or all female-producing; 65 flies were sleeved out in March and April on oak-trees which had proved successful in the previous year, and 16 of these yielded galls early in June. Unfortunately many of the galls had been attacked by parasites, greatly reducing the number of flies that hatched, so that the results, although not discordant with the last year's experiments, are of little value. The only considerable number obtained was 12 females from 19 galls produced by one parent. Mr. H. Scott has very kindly examined the external characters and mouth parts of a few male-producing and female-producing parthenogenetic females, and finds no recognisable differences between them except slight differences in size, which are probably accidental.

Maturation of the Eggs of the Spring (Parthenogenetic) Generation.

The part of the previous work which chiefly required completion and confirmation was that dealing with the maturation of the spring (parthenogenetic) eggs. It was found that in the eggs laid by some females polar divisions, or, at a later stage, groups of polar chromosomes, occurred, and, in the few cases in which the chromosomes of the segmentation mitoses could be counted in such eggs, the number appeared to be 10. In eggs laid by other females no polar chromosomes could be found, and these eggs showed 20 chromosomes where they could be counted in the segmentation division-figures. The number of cases observed was, however, not sufficient to substantiate this conclusion with certainty, especially since in some of the less well-preserved eggs the chromosomes were clumped together in the metaphase of the segmentation divisions, and might show in one part of the egg what appeared to be 10 or even fewer large chromosomes, while elsewhere in the same egg the full diploid number (20) was clearly present.

In the spring of 1910 I collected series of eggs from a large number of females, and from them obtained nineteen series of sections of well-preserved eggs, averaging about 27 eggs from each female, besides several smaller or less well-preserved series. Since the rate of development had proved rather variable in previous years, these were fixed at various ages, and not all the series show every stage from the undivided egg-nucleus up to segmentation divisions in which the chromosomes can be counted. Since, however, most of the series included eggs between two and six hours old, the majority show most of the stages desired, and several series are very complete. A comparison of the various series shows at once that they mostly fall into two groups: some show the diploid number (20) of chromosomes whenever the segmentation mitoses are clearly countable, and these never contain groups of polar chromosomes in the segmentation stages; others show the haploid number (10) in segmentation divisions, and in these a double or triple group of polar chromosomes can be found at the edge of the egg in nearly every specimen in which the sections are complete. Out of the nineteen series of eggs, five series are of the first type, containing diploid segmentation divisions and no polar chromosomes, while one other series, though containing only one egg with a recognisable mitosis, belongs to this type from the complete absence of polar chromosomes in the early segmentation stages. Of the second (haploid) type, seven series of eggs show undoubted polar mitoses or polar chromosomes; in two of these segmentation mitoses occur which are certainly haploid, and in two others, though the chromosomes cannot be counted accurately, the mitoses are almost certainly haploid. In the other three series there are no segmentation mitoses at a stage when the number of chromosomes can be estimated. Judged by the presence or absence of polar chromosomes, therefore, there are six series of the unreduced and seven of the reduced type; five of the former show diploid, two of the latter certainly haploid, segmentation mitoses. In the series of eggs collected in 1909 the proportion of the diploid type was probably greater. In the remaining six of the 1910 series all the eggs are too young to be classified with confidence. The fact that only two out of seven haploid series contain eggs with undoubted haploid segmentation mitoses, may seem insufficient evidence that eggs containing polar chromosomes have reduced segmentation nuclei, but it must be remembered: (1) that in none of the eggs with diploid mitoses can polar chromosomes be found, and (2) that every egg in which the sections are all complete shows polar chromosomes when the mitoses are haploid. In some of these the reduced number is so clear as to leave no doubt, while in a number of eggs of the diploid series the absence of polar chromosomes, even at a stage when there are only two segmentation nuclei, is equally certain. This will become clearer in the detailed account of the maturation and segmentation stages which follows.

In eggs belonging to both diploid and haploid series the early stages are alike. The nucleus appears as a rather small oval body, deeply and evenly stained, usually about half-way between the free end of the egg and the base of the stalk, either in the centre of the egg or nearer one side (Plate 17, fig. 1). It then approaches one side of the egg, at first with its long axis perpendicular to the egg-membrane, but as it reaches the edge it becomes round and finally flattened against the edge (figs. 2, 3). The nucleus then swells, and becomes an oval vesicle lying close to the edge with its long axis parallel to it, and an irregular spireme or network of chromatin bodies is now visible within it (figs. 4, 5). From this stage onward the behaviour of the nucleus in eggs of the two types is different. In eggs of the diploid series the nucleus in prophase of division as just described sinks for a short distance into the egg, and forms a mitotic figure parallel with the edge of the egg. I have only one egg in which the equatorial plate of this division is shown, and since it is lying sideways to the plane of the section the chromosomes cannot be counted accurately, but they are almost certainly more than ten. Several anaphase stages with the axis of the spindle parallel with the edge of the egg have been found: in one of these (fig. 6, *a*, *b*), in which the figure is cut in face in two successive sections, in one section 10 chromosomes are visible, while at the other a larger number appears, but it is not clear how many are cut so as to appear in both sections. Many cases have been found in which the first division is just completed, and two vesicular nuclei occur, both near the edge of the egg and lying in a line parallel with it. At first the nuclei are near together and are quite small (fig. 7); later they enlarge and are found at a greater distance apart. In several such eggs the series of sections is quite complete, and no trace of polar chromosomes can be found. The next division of these two nuclei appears usually to be perpendicular to the edge of the egg, and may be seen to show considerably over 10 chromosomes, even if they cannot be counted with complete accuracy. In the later divisions the 20 chromosomes can usually be counted most easily in anaphase (fig. 8, *cf.* fig. 46 of Part I of this paper); in some cases in prophase about 20 irregular meridional chromatic bands can be counted in the nucleus before the membrane disappears (fig. 9). In some later prophases the intra-nuclear origin of the greater part of the spindle is seen (fig. 10). Sometimes in metaphase in eggs which are not very well fixed the chromosomes become clumped together, and may give the appearance of about 10 or fewer very thick chromosomes. If care is not exercised such figures may be mistaken for haploid mitoses, but a little experience at once shows the difference, and usually in other parts of these eggs undoubted diploid mitoses may be found.

In all these cases no polar chromosomes can be found in any egg of the series to which they belong.

In the eggs of the haploid type the behaviour of the nucleus does not differ from that described until it has become flattened against the edge of the egg, and has then begun to swell, so that instead of being an evenly stained body it takes the form of an oval vesicle in which chromatic masses are distinguishable. Instead of sinking into the egg it continues to enlarge at the edge and occasionally a stage may be found in which the chromatin is aggregated at the two sides of the nucleus, one mass being next to the edge of the egg and the other towards the inside (fig. 11). As was described in the previous part, both for the summer and spring eggs, no regular equatorial plate has ever been found. The next stage, of which I have a number of examples, shows the chromosomes separating into two groups; an inner in which they appear as a group of parallel rods as in an ordinary anaphase, and an outer in which they are very irregularly arranged in a loose group close to the edge of the egg.

In some sections of this stage (fig. 12) it can be seen that the inner group of parallel chromosomes is in the equator of an elongated mitotic spindle, and although I have no very clear figures of these stages, those which I have suggest that the rod-like chromosomes divide transversely, giving rise at the inner end of the spindle to the egg-nucleus, at the outer end to a second group of polar chromosomes (fig. 13, *a*, *b*, *cf.* Plate 3, fig. 42 of first part of this paper).

In this way a large outer and smaller inner group of polar chromosomes arise, and during the segmentation stages two groups are often found, one of which frequently contains about 10 chromosomes. In other cases the outer group appears to divide into two, both of which are irregular and can never be counted with accuracy. Fig. 14, *a*, *b*, represents the only case in which I have found this division taking place. On the whole it is clear that the polar divisions of the spring eggs which undergo maturation do not differ widely from those of the summer eggs, which require fertilisation, as described in Part I. There is in both essentially a double polar division, in the first part of which an irregular outer group of chromosomes separates from a more regular inner group, followed immediately by the division of the inner into egg-nucleus and inner polar group, each of which contains the reduced number of chromosomes. The whole process is so nearly simultaneous as to give the impression of a single division, a fact which led me to hazard the suggestion in Part I of this paper that only one maturation occurred in the parthenogenetic eggs. The fresh material since collected, however, shows that the inner chromosome group does undoubtedly divide just after its separation from the outer, and the phenomena must therefore be interpreted, as in the summer eggs, as a modified double polar division. The maturation thus differs rather in detail than in essence from what occurs in all other Hymenoptera in which the polar divisions have been examined.

After the polar divisions, the egg-nucleus sinks into the yolk and almost immediately forms the first segmentation spindle, so that the prophase of this mitosis may not rarely be found in the same section with the polar chromosomes. The axis of the first segmentation spindle appears usually to lie nearly, but not quite, parallel to the long axis of the egg; after the division is completed, the daughter nuclei travel widely apart, so that the division-figures of the second segmentation mitoses are usually widely separated. In these and the later segmentation divisions the chromosomes are most easily counted in anaphase; in metaphase they may be clumped, but, even so, differ from the clumped diploid groups described above in their smaller size. In anaphase figures cut across it is possible to count 10 with confidence in some cases (fig. 15), and in a number of others to see clearly that the number is undoubtedly much less than 20, though not accurately countable (figs. 16, *a*, *b*; 17, *a*, *b*). In a few cases one chromosome appears to lag very much behind the others, as if it was being left out of the nucleus altogether, but in other eggs I have not seen anything of the kind, even when mitoses of the same stage are present. During the segmentation mitoses, the groups of polar chromosomes persist at the edge of the egg (fig. 18), but when the nuclei come to the surface to form the blastoderm, they are no longer recognisable. The chromosomes and mitotic figures in the blastoderm divisions are too small and crowded for counting to be possible.

Later Stages: Segmentation and Blastoderm.

I have no great number of sections of eggs in the later segmentation and blastoderm stages, but among them are some which show phenomena of interest. In eggs which have a well-developed blastoderm, a number of nuclei remain in the yolk, and these are often of two types in the same egg. There are a number of rather large vesicular nuclei, sometimes showing a faint reticulum, and, in the others, a clear space inside the membrane enclosing a more compact stained mass, rather suggesting that the nuclear contents have contracted. Among these there are frequently many smaller nuclei, either evenly stained throughout or consisting of a deeply stained mass surrounded by a narrow clear space. When the nuclei of the blastoderm are dividing, which they all do simultaneously, they show clearly defined, though very small and compact, mitotic spindles; the nuclei of the yolk in the same egg show no spindles, and strongly suggest amitotic division, for various stages can be found, from nuclei which are simply elongated to two or sometimes three nuclei in which the membranes are in contact (figs. 19, 20, *a*, *b*). Stages of this apparent amitotic division are found in the yolk-nuclei of most eggs in the blastoderm stage, whether the blastoderm-nuclei are undergoing division or not. In advanced blastoderm stages below the layer of blastoderm-nuclei there is a belt of protoplasm with no yolk, and inside this the yolk fills the centre of the egg, and contains all the yolk-nuclei, none being found in the protoplasmic belt.

In two large series of eggs (each laid by a single female) a number of later segmentation stages are represented preceding the formation of the blastoderm. Unfortunately, neither of these series contains any egg which shows conclusively whether it is haploid or diploid. Both contain eggs showing bodies at the edge which might be remnants of the polar chromosomes, but which cannot be identified as such with certainty. In some eggs, but not in all, of both series, very large nuclei occur, among which are scattered others of the normal size. In late segmentation stages these large nuclei are sometimes found in division; the figures are obscure, possibly owing to imperfect fixation, but appear to be diploid. Divisions of the smaller nuclei, on the other hand, suggest haploid figures, but I have none in which the chromosomes can be counted (fig. 21, *a*, *b*). In other eggs, some of these large nuclei are either undergoing amitotic division or are being formed by fusion of two or more smaller nuclei; groups of two, three, or even more nuclei are found in close contact, while here and there are large, apparently single nuclei as big as the group of smaller ones (figs. 22, 23, 24). In some eggs among these are nuclei which are drawn out into processes, or have an irregular shape (fig. 25). At this stage the distinction between yolk- and blastoderm-nuclei cannot be made, for all are scattered indiscriminately through the egg; they are most numerous at the lower end of the egg, towards the stalk. In eggs of other series the nuclei are all alike at this stage, and show nothing unusual, but that the two series described are not entirely exceptional is shown by the occurrence of one or two similar eggs among later segmentation stages collected in 1908. That the phenomena are due entirely to bad preservation appears improbable. From incomplete observations such as these it would be premature to draw definite conclusions, but it may be suggested provisionally that during the later segmentation stages the nuclei become differentiated into two kinds; some will form the blastoderm and continue to divide normally by mitosis, others remain as yolk-nuclei and show signs of degeneration in the frequent massing together of their chromatin into a single, large, deeply-staining mass, in their great variation in size, possibly due to fusion of nuclei in some cases, and lastly, in dividing irregularly by amitosis. I find that such "yolk-nuclei" exist also in the blastoderm stages of the summer (fertilised) eggs, and these show signs of degeneration and indications of amitosis similar to those found in the spring eggs. In an embryo of the summer generation fixed when three days old, the yolk is already largely absorbed and the yolk-nuclei appear to be undergoing atrophy. Whether these suggestions are correct can only be determined by further work on fresh material collected for the purpose.

Ovarian Mitoses of Parthenogenetic Females.

In the spermatocyte divisions of the male, I described (Part I, pp. 92, 94, and 95) a small extra-nuclear body which does not divide with the cell, and is, therefore, present in only half the spermatids. It seemed not impossible that the presence of this body in half the spermatozoa might determine whether the fertilised egg developed into a male-producing or female-producing parthenogenetic individual. To recognise such a body in the fully grown egg would be almost impossible, owing to the quantity of yolk, but it seemed just possible that it might occur in the oogonial mitoses of some of the parthenogenetic females. I have cut sections of the ovaries of seven larvæ of the parthenogenetic generation, in the hope of finding it, and in five of them oogonial mitoses occur in which no such body is visible. In the other two no oogonia are in course of division. It is possible, of course, that all these larvæ would have developed into female-producing individuals, but since the extra-nuclear body is not recognisable in the spermatogonia in the male larva, it is more likely that if such a body is present in the female at all it is not to be found at so early a stage.

Mitoses in the Nervous System.

In a footnote added to the first part of this paper while in proof (Part I, p. 91) I mentioned that in the developing nervous system of male larvæ mitoses occurred with the haploid number (10) of chromosomes. Examples of this are shown in figs. 26, 27, 28, *a*; I have not much to add to my earlier account. I have found undoubted haploid figures in the nervous system of six male larvæ, and in no male examined are there certainly no such mitoses; in a few, no countable mitoses could be found. In addition to the haploid mitoses I have occasionally found diploid figures (figs. 28, *b*; 29); and these appear to be in cells of a different kind. In the younger larvæ there appear to be cells of two kinds in the developing nervous system, some with numerous faint chromatic bodies in the nucleus, and a very small nucleolus, which from their appearance and distribution are probably true nerve-cells; others, more variable in size, with one or two large conspicuous nucleoli, which occur chiefly round the edge of the nervous system or in groups at a deeper level. These latter cells resemble the mesoderm cells of the larva, and the diploid mitoses appear to occur in them; possibly therefore they are immigrant mesoderm cells forming supporting tissue. In female larvæ, of both spring and summer generations, I have found only diploid mitoses in the nervous system as elsewhere.

Abnormal Nuclear Divisions in the Larva.

In the footnote referred to above, in addition to the haploid divisions in the nervous system of the male larva, I mentioned that in larvæ of both sexes giant nuclei occur below the hypodermis, some 15 or 20 μ in diameter, and that in the single mitosis which I had seen among these nuclei (an anaphase, fig. 30) there were at least 50 chromosomes at each pole. I have found no further divisions of this kind, and have made no full observations on the nature of the nuclei among which it occurs. They appear, however, to be in connection with developing muscle-fibres, between the hypodermis and the fat-body, and in younger larvæ they seem clearly to be the nuclei of the cells from which muscles are developing. The cells of the fat-body have also very large nuclei, which resemble those of the muscle cells rather closely, but usually have a less fine and regular distribution of the chromatin. Similar nuclei occur in connection with the developing muscles of the spring generation, but I have not found any in mitosis.

Summary.

The more important observations recorded are as follows:—

1. There are two kinds of parthenogenetic females in the spring generation of *Neuroterus lenticularis*, which lay eggs differing in their behaviour as regards maturation.

2. In the eggs laid by one class of female there is no maturation division; the nucleus comes to the surface, reaches the stage of prophase of division, then sinks in for a short distance and divides by a mitotic spindle parallel with the egg-margin. In such eggs early segmentation divisions show the diploid number (20) of chromosomes, and no polar chromosomes are ever found.

3. In eggs laid by the second class of female the preliminary behaviour

of the nucleus is similar to the former. Instead, however, of sinking in, it divides at the surface by a division perpendicular to the edge, producing an irregular outer group of chromosomes (first polar nucleus) and an inner group of parallel rod-like chromosomes. The latter divide immediately, apparently transversely, into an inner group which forms the egg-nucleus, and an outer or second polar group. The first polar group may divide into two. In the early segmentation mitoses of eggs of this class the haploid number (10) of chromosomes is found, and in complete series of sections of such eggs a double or triple group of polar chromosomes is always found at the edge of the egg.

4. Since it is known that some parthenogenetic individuals lay eggs which all develop into females, and others lay only male-producing eggs, and since the female shows the diploid chromosome number in all its cells, while the male has the haploid number in the spermatogonia and nerve-cells, it is suggested that the eggs which undergo no maturation division become females, those which undergo reduction males.

5. In the later segmentation stages of some eggs, and commonly in the yolk-nuclei of eggs which have reached the blastoderm stage, phenomena are described which are interpreted as indicating amitotic division.

6. The haploid mitotic figures in the nervous system of the male, referred to previously, are more fully described, and a case is mentioned of a mitosis in a developing muscle-cell with about three times the normal (diploid) number of chromosomes.

General Considerations.

The facts set forth above confirm the conclusions with regard to sex-determination in Neuroterus which were drawn provisionally in the first part of this paper. There is no need, therefore, to discuss them further. I attempted, however, in my previous discussion to link the phenomena of sex-determination in Neuroterus on to the results obtained by various methods in other groups of animals, and formulated a general scheme of sex-production, on the lines of Mendelian heredity, in which Neuroterus formed a special case. This scheme consisted in the assumption that in general the female contains female and male sex-determinants, represented by the symbols ♀ and ♂, which segregate in oogenesis so that two kinds of eggs are produced, bearing ♀ and ♂ determinants respectively. The male contains only the ♂ determinant, but is heterozygous in the sense that in its unreduced germ-cells only one ♂ determinant is present, which goes into half the spermatozoa, the other half of the spermatozoa containing no sex-determinant. This was expressed by representing the male as ♂ ⊙, producing ♂-bearing and ⊙-bearing spermatozoa (the latter being those without sex-determinant). Selective fertilisation was now supposed to occur, ♀-bearing eggs being fertilised by ♂-bearing spermatozoa giving females (♀ ♂), ♂-bearing eggs by ⊙-bearing spermatozoa giving males (♂ ⊙).

It was pointed out that while such cases as *Abraxas grossulariata*, the cinnamon canary, and several breeds of fowls prove that the female is heterozygous in respect of sex, it is also clear that in some cases the male is in some sense heterozygous, as is proved by the inheritance of colour-blindness and other affections in Man. Since this was written, a new and remarkable case has been described by Morgan in Drosophila,* in which the heterozygous condition of the male is proved in exactly the same way as that of the female is shown in Abraxas. Now it is known from the work of Miss Stevens† that the male of Drosophila has a pair of unequal idiochromosomes, and thus produces two kinds of spermatozoa, and a recent paper by Guyer‡ shows that in Man also two kinds of spermatozoa are formed. It appears, therefore, that in both the best-established cases in which the heterozygous condition as regards sex of the male has been shown by experiment and observation in heredity, the male is also heterozygous in respect of chromosomes, and produces two visibly different kinds of spermatozoa. In Abraxas, no such difference in the chromosomes of the spermatozoa is to be found,§ nor has it been found by Miss Stevens or Miss Cook in other Lepidoptera.‖ These facts naturally suggest that in some species the male is heterozygous as regards sex, in others the female, and tend to support the suggestion of Wilson,¶ Castle,** and Morgan,†† that specific male and female sex-determiners do not exist, but that femaleness consists in the presence of some extra factor superposed on what would otherwise produce maleness. If the female receives this factor from both parents, the male from only one, the female would appear homozygous as regards sex, the male heterozygous; if the female receives it from one parent and the male lacks it altogether, the female is heterozygous and the male homozygous. This scheme is perfectly satisfactory as long as no case is known in which there is evidence from breeding experiments that both the male and female are heterozygous. Hagedoorn* has described such a case in fowls, but his account is not very clear and no details are given. It indicates that a heterozygous female, paired with a homozygous recessive male, transmitted the dominant character only to its male offspring; the heterozygous males so produced transmitted, in the next generation, the dominant character only to their female children. This, if correct, can only be explained on the assumption that both sexes are heterozygous for the sex-determinants, and that the dominant character is always associated in this instance with the determinant which I have represented by the symbol ♂. The hypothesis of the American writers would then fall to the ground.

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* 'Science,' July, 1910, vol. 32, p. 120.

† 'Journ. Exp. Zool.,' vol. 5, p. 365.

‡ 'Biological Bulletin,' 1910, vol. 19, p. 219.

§ Doncaster, 'Proc. Camb. Phil. Soc.,' vol. 16, pt. 1, p. 44.

‖ N. M. Stevens, 'Carnegie Inst., Washington, Publ.,' 1906, 36, Part 2, p. 48; Cook, 'Proc. Acad. Nat. Sci., Philad.,' 1910; summarised, 'Zentralbl. f. Allg. u. Exp. Biol.,' 1910, p. 620.

¶ 'Science,' January, 1909, vol. 29, p. 53.

** 'Science,' March, 1909, vol. 29, p. 395.

†† 'Journ. Exp. Zool.,' 1909, vol. 7, p. 332. See also 'Amer. Naturalist,' vol. 45, No. 530, p. 65, which has appeared since the above was written.

Apart from this case, there is no certain means of deciding between the two hypotheses; that of Wilson, Castle, and Morgan is simpler in not requiring selective fertilisation, for which little evidence exists; but until many more cases of sex-limited inheritance are known, and it is found that they never indicate that the heterozygous condition can exist in both sexes of the same species, both hypotheses accord equally well with the known facts. One further possibility should be mentioned, which does not differ greatly from that suggested by Morgan in the paper referred to above, and also more recently by Montgomery.† This is that the "factors" which have been referred to as sex-determiners are not really such, but that every individual contains potentially the character of both sexes—is in fact potentially hermaphrodite—but for the appearance of one or other of these characters additional factors are required. If the factor which I have called the ♀ determinant is present, it brings out the female characters and suppresses the male, if the ♂ determinant is present in the absence of the ♀, it brings out the male characters. On such a hypothesis as this the results arrived at by Geoffrey Smith‡ would be explained. He infers a "sexual formative substance" in Malacostracan Crustacea, which causes the appearance of female characters; when a male is parasitised by Sacculina the parasite induces the secretion of such a substance by the organism, and the animal assumes the female characters and may even produce eggs in its testis. Even though such a male might, on the hypothesis summarised above, have the constitution ♂ ⊙, *i.e.* might not contain the factor which brings out the female characters, yet the secretion induced by the parasite might have the same effect as this factor, and thus cause the female characters to appear. This hypothesis of potential hermaphroditism of both sexes, combined with special ♂ and ♀ activators, would thus allow of the occasional modification of sex by environment, if the stimuli supplied were able to bring about the same physiological effects as the inherited ♂ and ♀ determinants. That environment may have some such effect in modifying somatic characters which are inherited alternatively is indicated by the work of Tower,* and there seems to be no *a priori* reason for denying such an effect in the case of sex.

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* 'Archiv f. Entwicklungsmechanik,' 1909, vol. 28, pp. 18, 26.

† 'Biological Bulletin,' 1910, vol. 19, p. 9.

‡ "Studies in the Experimental Analysis of Sex," 'Q.J.M.S.,' 1910, vol. 55, p. 225.

EXPLANATION OF FIGURES ON PLATE 17.

All the figure are freehand drawings, made with a Zeiss aprochromat. 3 mm., 1·40 n.a., and compensating ocular 12.)

FIGS. 1—3.—Three stages of the approach of the nucleus to the edge of the egg, preceding maturation.

FIGS. 4, 5.—Prophases of first segmentation division ; diploid type of egg.

FIG. 6 (*a*, *b*).—First segmentation division, same series as figs. 4, 5 ; *a* and *b* show the mitotic figure cut in two successive sections.

FIG. 7.—Completion of first segmentation division ; same series as figs. 4—6.

FIG. 8.—Anaphase group of segmentation mitosis, diploid type. Nineteen chromosomes are visible in this section ; at the other end of the same spindle, two sections removed, 15 or 16 can be counted.

FIG. 9.—Prophase of diploid segmentation division, showing about 20 chromosomes arranged in irregular meridional bands under the nuclear membrane.

FIG. 10.—Late prophase, same series as fig. 8, showing intranuclear origin of spindle.

FIG. 11.—Prophase of polar division, haploid type of egg.

FIG. 12.—First polar division, nearly completed. The division figure ran obliquely through two sections ; all the inner part is drawn from one section, the greater part of the outer group of chromosomes from the next.

FIG. 13 (*a*, *b*).—Second polar division ; the division figure runs obliquely through two sections. *a* represents the separation of the chromosomes of the egg-nucleus from the second polar chromosomes ; *b*, in the next section, the outer polar group.

FIG. 14 (*a*, *b*).—Two successive sections, showing completion of second polar division. In *a*, egg-nucleus on right, second polar group in middle, part of division spindle of outer polar group on left ; *b*, division-figure of outer (first polar) group.

FIG. 15.—Anaphase group of segmentation division, haploid type.

FIG. 16 (*a*, *b*).—Anaphases of haploid segmentation divisions ; *a*, cut across ; *b*, seen sideways. Both from the same egg.

FIG. 17 (*a*, *b*).—Two late anaphases, haploid type, from the same egg. In *a* only the chromosomes at the upper side of the figure are shown. In *b* all the chromosomes (8) visible in the section are shown ; the other end of the figure was broken.

FIG. 18.—Group of polar chromosomes during segmentation stages ; same egg as fig. 15. To the left, a large group of about 20 (outer polar group) ; to the right, smaller inner polar group.

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* 'Biological Bulletin,' 1910, vol. 18, p. 285.

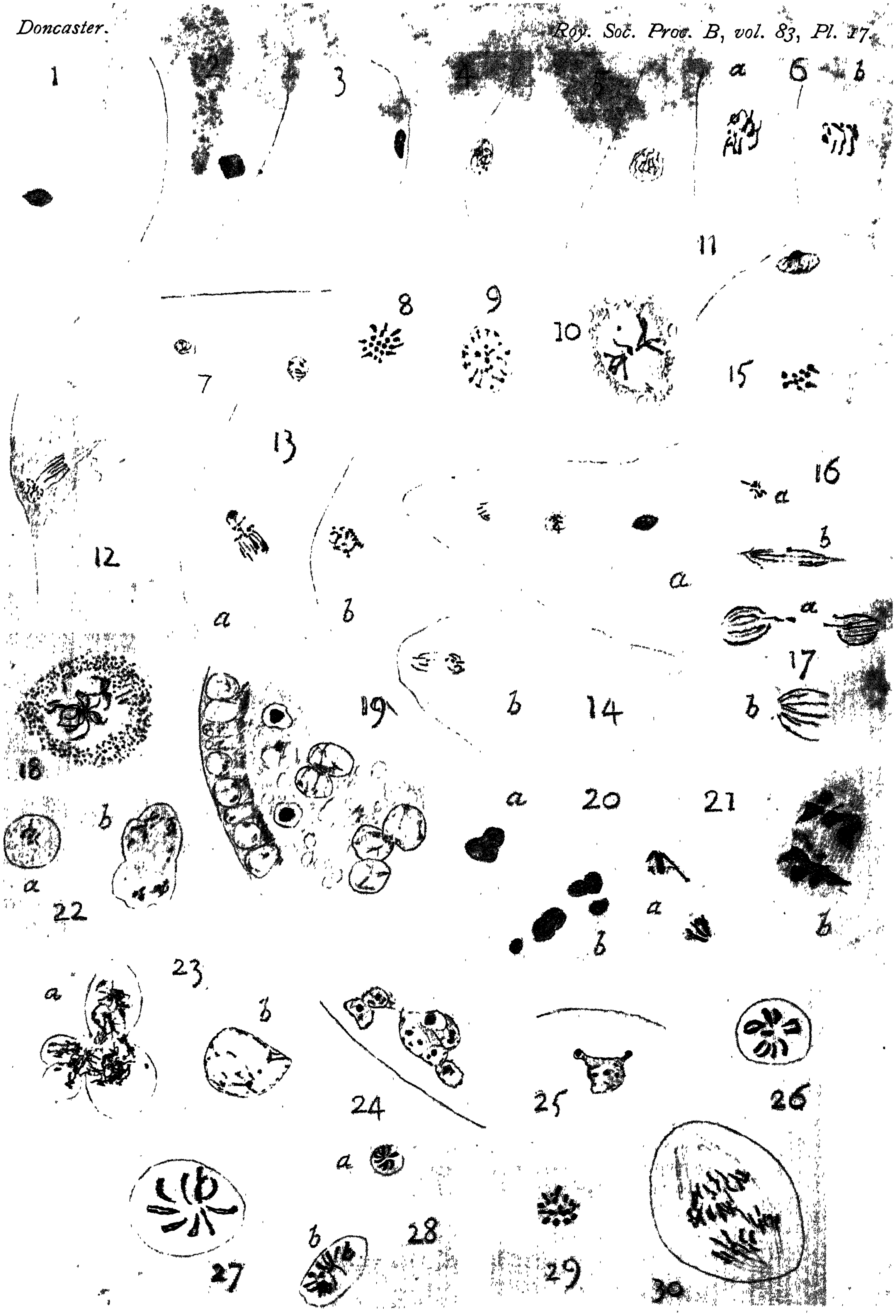
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Fig. 19.—Part of an egg in blastoderm stage, showing two groups of vesicular nuclei apparently undergoing amitotic division and two smaller nuclei with dark mass in the centre.

Fig. 20 (*a*, *b*).—Stages of apparent amitotic division in blastoderm stage. *a*, early stage; *b*, a group of nuclei from another part of the same section.

Fig. 21 (*a*, *b*).—Division-figures in an abnormal egg. *a*, division of smaller nucleus suggesting haploid type; *b*, division of larger nucleus in the same egg.

Fig. 22 (*a*, *b*).—Nuclei in an egg from same series as fig. 21. *a*, ordinary nucleus; *b*, large nucleus undergoing amitotic division or fusion.

Fig. 23 (*a*, *b*).—*a*, amitotic division or compound fusion; *b*, single nucleus from same egg. Same series as figs. 21, 22.

Fig. 24.—Group of nuclei (amitosis or fusion). The two nuclei to the left are drawn from the next section to the larger group. Same series as fig. 20.

Fig. 25.—Nucleus showing irregular shape. Same egg as fig. 24.

Figs. 26, 27.—Haploid divisions (metaphase) in nervous system of male larvæ (summer generation). In fig. 27 each chromosome is seen split preparatory to division.

Fig. 28 (*a*, *b*).—Two equatorial plates in the nervous system of male larva. *a* is haploid (9, or possibly 10, chromosomes visible); *b*, diploid (about 16 visible).

Fig. 29.—Diploid group, nervous system of male larva (same specimen as fig. 28). Eighteen chromosomes are visible.

Fig. 30.—Mitosis of giant nucleus in muscle-cell, female larva, summer generation. The figure extends through two sections, only one of which is represented. Less than half of the chromosomes are shown.

On the Leaves of Calamites (Calamocladus *Section*).

By H. Hamshaw Thomas, M.A., Curator of the Botanical Museum, Cambridge.

(Communicated by A. C. Seward, F.R.S. Received December 2, 1910,—
Read February 2, 1911.)

(Abstract.)

In spite of the great progress which has been made in the study of Coal Measure plants, the subject of leaf structure has been largely neglected. The author is investigating the structure of the leaves of the principal groups of Palæozoic plants with the view of determining their morphological and biological characters, and also of obtaining some knowledge of the conditions under which they grew.

The specimens of Calamite leaves described in this paper have been found chiefly in slides in existing collections, and most of the material originally came from the Halifax Hard Bed of the Lower Coal Measures.

Most of the leaves found, were very small, being only 1 to 3 mm. long and 0·8 to 1 mm. broad. They are falcate in shape, and were borne on slender twigs in alternating whorls of four. The structure of these slender twigs differs somewhat from that of the young Calamitean stems already described by Williamson and others, but it may be compared in some features with the structure of the young stems of some modern Equisetums.

The tissues of the small leaves show a concentric arrangement. In the centre there is a vascular bundle consisting of four or five small tracheides surrounded by thin-walled elongated cells. The bundle is surrounded by a zone of cells with dense black contents, termed by the late Thomas Hick the melasmatic tissue, which is probably comparable with the bundle-sheath of the leaves of modern plants. The cells of the palisade-like assimilating tissue abut on this, and have large spaces between them. The epidermis is thinner on the concave side of the leaf, and the stomata are confined to this face. They are characterised by transversely striated guard cells, similar to those seen in many species of modern Equisetums. The leafy twigs just described seem to be identical with the impression-species *Calamocladus charaeformis* (Sternb.). Their structure seems to indicate that they grew in a pendulous manner. Specimens have been obtained showing variations in structure from the normal type.

Four other types of leaf have been discovered, differing in size and in arrangement of tissues. In all of these there is a very conspicuous strand of sclerenchymatous fibres running up the adaxial side of the leaf and

forming a large part of its apex. These fibres become more conspicuous in the longer leaves. In some types the thin-walled (phloem) tissue of the bundle is much reduced, or even absent. The melasmatic tissue also varies considerably in amount. Some of these longer leaves were probably identical with *C. grandis* (Sternb.), others with *C. equisetiformis* (Schloth.). They are characterised by a more compact structure with smaller and fewer intercellular spaces.

The structure of the smaller leaves probably indicates that they grew in a moist situation, or where the atmosphere was humid. The larger leaves are more xeromorphic in character. The results obtained from this work indicate that the Calamites were in all probability truly microphyllous.

The Action of the Venom of Echis carinatus.

By Sir THOMAS R. FRASER, M.D., LL.D., Sc.D., F.R.S., Professor of Materia Medica, University of Edinburgh; and JAMES A. GUNN, M.A., M.D., D.Sc., Assistant in the Materia Medica Department, University of Edinburgh.

(Received January 27,—Read March 16, 1911.)

(From the Pharmacology Laboratory of the University of Edinburgh.)

(Abstract.)

The venom used consisted of the liquid venom, which had been rapidly dried immediately after it had been taken from living serpents in India by the process of "milking." Its minimum lethal dose, by subcutaneous injection, per kilogramme, was found to be for the frog, 0·009 gramme; for the rabbit, 0·0009 grm.; for the guinea-pig, 0·001 grm.; for the rat, 0·00075 grm.; for the cat, 0·008 grm.; for the pigeon, 0·004 grm.; and, by intramuscular injection, for the pigeon, 0·001 grm.

In the case of all these animals, the cardinal symptoms, in order of time and of prominence, are hæmorrhages occurring locally and distally, feebleness of the circulation and anæmia, arrest of respiration and loss of reflex excitability, and arrest of the heart in diastole.

No evidence could be obtained, either in cold- or warm-blooded animals, of a direct action of the venom on the brain or spinal cord. At a time when there occurs definite impairment of the functions of the central nervous system, this can adequately be accounted for by the coincident or

pre-existent deterioration of the quality of the blood and the feebleness of the circulation. The venom has, moreover, no effect on the nerves or nerve terminals of voluntary muscle. The venom has no appreciable effect on skeletal muscle. The effects on the circulation are produced partly directly and partly indirectly. From the point of view of lethality, the indirect effects, due to blood changes, are the more important.

When perfused through the frog's heart, strong solutions bring about an increase in the rate, followed by arrest of the heart in systole, and weaker solutions also quicken the heart, but arrest it in diastole. The venom also renders the heart-wall permeable to the contained blood solution, an effect apparently due to separation of the cardiac muscle fibres.

Solutions of the venom diminish the flow through the frog's blood-vessels, when perfused through them; but this effect is due, not to a direct constricting action on the vessels, but to the production, by the hæmorrhagin contained in the venom, of an intense œdema of the tissues of the frog.

In mammals, even very small doses of the venom injected intravenously may rapidly arrest the circulation by the production of intravascular clotting. If intravascular clotting be prevented, the venom produces a fall of blood-pressure, due partly to slowing, but mainly to weakening, of the heart's contractions.

Echis venom has no appreciable effect on the lymph hearts of the frog.

The toxic effects of the venom are due chiefly to the production of alterations in the quality of the blood, of which the following are the most important:—Especially with lethal doses, there occurs a great reduction in the number of the red blood corpuscles in the circulating blood, which is due chiefly to hæmorrhages, but, in the case of large intravenous doses, is due also to a small amount of hæmolysis. The number of the leucocytes is usually considerably increased for some days after subcutaneous injection. The hæmolytic action of the venom is unimportant in producing its lethal effects, and death frequently occurs without any hæmolysis. The venom has a moderate hæmolytic action on the blood *in vitro*. Positive and negative phases of blood coagulability are produced by Echis venom in a manner similar to that which has already been described in the case of several venoms.

Echis venom exerts little, if any, direct action on the respiratory centre or on the terminations of the phrenic nerves. Non-lethal doses of the venom produce a rise of temperature; lethal doses a rise, followed by a fall, of temperature. Lethal doses cause a diminution in the total quantity of urine secreted, and produce albuminuria, and, frequently, hæmaturia.

The Chemical Dynamics of Serum Reactions.

By Captain A. G. McKendrick, I.M.S., Assistant Director, The Pasteur Institute of Southern India, Coonoor.

(Communicated by Sir James Dewar, F.R.S. Received November 30, 1910,—
Read March 30, 1911.)

A multitude of phenomena of widely varying character is seen in experimenting with blood sera: Agglutination, bacteriolysis, hæmolysis, precipitin, opsonin, and stimulin reactions, and others occur.

In the serum two elements are distinctly recognisable: (1) A so-called *amboceptor*, which is characterised by its being able to withstand comparatively high temperatures, and by the fact that it can act alone.

(2) A so-called *complement*, which is put out of action by comparatively low temperatures, and which cannot act unless the amboceptor has previously acted.

It is the object of this paper to show that the above reactions are subject to the law of mass action, and that they can be expressed by the whole or part of the equation

$$\frac{dz}{dt} = \frac{y}{c}\left(\frac{x}{cz} - z\right) - \left(\frac{y}{c} - z\right)^2, \tag{1}$$

according as both elements are present, or only amboceptor is present.

y = per cent. of amboceptor,
x = " complement,
c = a constant of dilution,
z = a complex quantity, denoting effect, as observed in any of the above reactions,
t = time.

This formula merely states that amboceptoral action is bimolecular, and that complement action is monomolecular, under the influence of a catalytic effect of the amboceptor.

Writing the equation thus:

$$\frac{dz}{dt} = \frac{y}{c}\left(\frac{x}{ca} - z\right) - \left(\frac{y}{c} - z\right)^n \tag{2}$$

is the mathematical form of stating the characters of the two components (as given above), with the hypothesis that amboceptoral action is directly opposed to complement *plus* catalyst action—not a direct opposition in the fluid, but an opposition in their action on the cell.

$\frac{y}{c} - z$ = concentration of free amboceptor,

$\frac{x}{ca} - z$ = concentration of free complement,

$\frac{y}{c}$ = concentration of amboceptor, both free and combined (*i.e.* the concentration in which it acts catalytically).

The fundamental equation (1) was obtained from a study of the value of z got by mixing varying proportions of y and x.

The details of experiment are as follows:—The materials used were human serum for the serum components, and the cells were washed horse cells. Three fluids were made up:—

	Unheated serum.	Heated serum.	*i.e.* y.	x.
	parts.	parts.		
Fluid *a*	4	0	4	4
„ *b*	2	2	4	2
„ *c*	1	3	4	1

A series of dilutions of each of these fluids was put up as follows:—

	Fluid *a*, *b*, or *c*.	0·85-per-cent. NaCl solution.	Washed cells.	*I.e.* per cent. amboceptor.
	c.c.	c.c.	c.c.	
1	2·45	0·0	0·05	98·0
2	2·05	0·4	0·05	82·0
3	1·8	0·65	0·05	72·0
4	1·55	0·9	0·05	62·0
5	1·3	1·15	0·05	52·0
6	1·05	1·4	0·05	42·0
7	0·8	1·65	0·05	32·0
8	0·55	1·9	0·05	22·0
9	0·5	1·95	0·05	20·0
10	0·44	2·01	0·05	17·6
11	0·375	2·065	0·05	15·0
12	0·31	2·14	0·05	12·5
13	0·25	2·2	0·05	10·0
14	0·187	2·263	0·05	7·5
15	0·125	2·325	0·05	5·0
16	0·0625	2·3875	0·05	2·5
17	0·0	2·45	0·05	0·0

I used this arrangement of combinations of heated and unheated sera because by it the area in which the phenomenon of diversion occurs, whose characteristics I had set out particularly to investigate, can be explored and disclosed to view. The tubes were incubated at 37° C. for one hour, and were placed on ice overnight.

The observed results are given in Table I for the lowest complete lysis, in Table II for the lowest partial lysis. The first two columns give observed values, and the third column contains the corresponding values calculated by means of the fundamental equation, which will be referred to later.

Table I.—For Complete Lysis.

y and x varying; z constant; $dz/dt = 0$ (*i.e.* when the reaction has ceased). $c = 11·37$, $z = 1·256$ (the method by which these constants were obtained will be explained at the end of this section after the mathematical discussion).

y.	x observed.		x calculated.
per cent.	per cent.	per cent.	per cent.
5·0			39·54
10·0			20·23
14·281*	>14·0		17·936
20·0		20·0	19·54
30·0		<30·0	28·27
40·0		<40·0	38·72
50·0		<50·0	49·98
52·5		<52·5	52·88
55·0	about 55·0		55·85
62·5	>62·5		64·66

* "Point of diversion."

Table II.—For Lowest Partial Lysis.

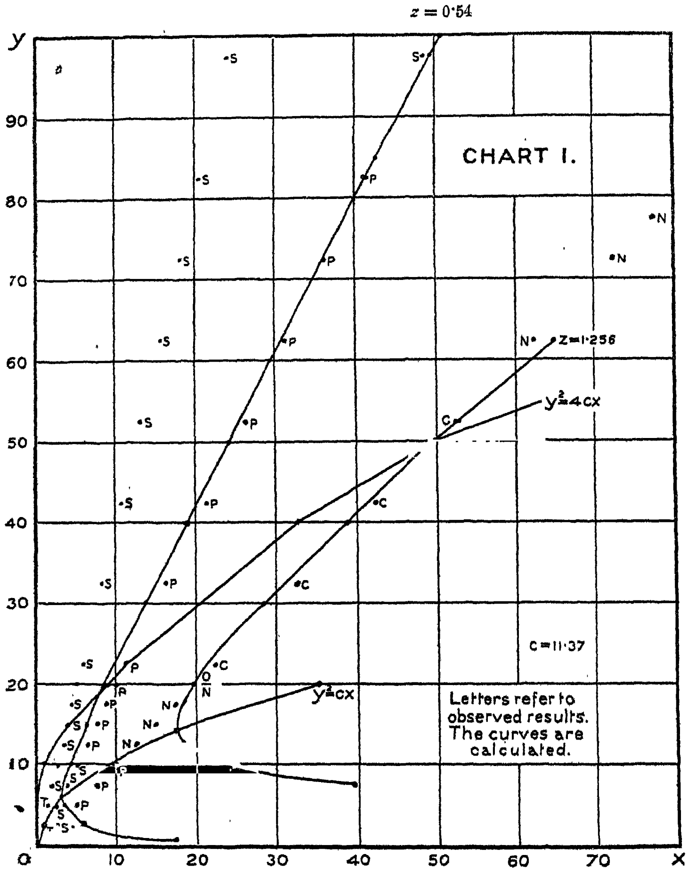
Conditions as in Table I. $c = 11·37$, $z = 0·54$.

y.	x observed.		x calculated.
per cent.	per cent.	per cent.	per cent.
1·0			17·59
2·5			6·06
5·0	>2·5	<5·0	3·45
6·1398*			3·31
10·0	>5·0		4·22
15·0	>3·75	< 7·5	6·14
20·0		<10·0	8·50
30·0	>7·5	<15·0	13·56
40·0		<20·0	18·79
50·0		<25·0	24·09
82·5	about 41·25		41·48
85·0	about 42·25		42·72
97·5	>48·75		49·54
100·0			50·89

* "Point of diversion."

The results of this experiment are shown in Chart 1, where points—

Marked C indicate complete lysis.
„ N „ nearly complete lysis.
„ P „ partial lysis.
„ S „ slight „
„ T „ a trace of lysis.



It is particularly to be noticed that the curves are thrown back (towards the right) in their upper parts. This exhibits the special phenomenon (already referred to) called "complement diversion." As the curves appeared to form a regular series, I considered that the principle lying at the root of serum reactions was to be found in this phenomenon.

Assuming the curves to be hyperbolic from their appearance when z was constant, I looked for the asymptotes. One asymptote was obviously $y = 0$; another suggests itself as of the form $x/z - y = 0$; for, letting z

take smaller and smaller values, this asymptote approached closer and closer to $x-0=0$. At the same time, it was clear that, in general, these inclined asymptotes cut the axis of y at some point other than the origin; and, on the other hand, this point of intersection reached the origin when z became zero that is, there was no lysis in the absence of both amboceptor and complement. I chose, then, as the final equation for this asymptote,

$$\frac{x}{z}-y+cz=0.$$

Hence the equation of any of these curves, in which z was constant, became

$$y\left(\frac{x}{z}-y+cz\right)=d, \tag{3}$$

where d was in general some function of z, to be determined.

The experimental curves showed that the lytic value for any one of these curves could not be attained if the complement fell below a minimal amount, depending on the constant value of the lysis for the particular curve in question. Seeing that the phenomenon of "diversion" begins here, the corresponding point on any hyperbola may appropriately be called its "point of diversion." At a point of diversion the tangent to the hyperbola is parallel to the axis of y; hence to find these points we have only to find x and y from equation (3), and from the equation got from putting $dx/dy=0$ derived from it when z, and therefore d, are constant. This latter equation is

$$\frac{x}{z}-2y+cz=0. \tag{4}$$

Whence, by means of (3), we get at once

$$y=\sqrt{d}, \quad \text{and} \quad x=2z\sqrt{d}-cz^2 \tag{5}$$

the co-ordinates of the "point of diversion" in terms of z.

The co-ordinates of the "point of diversion" of any of these hyperbolas satisfy equation (4) for $z=$ constant. The envelope of this line, for variations of z, is

$$y^2=cx. \tag{6}$$

If we assume that all "points of diversion" lie on this parabolic cylinder—a view which, at least, seems favoured by the appearance of the curves—we are able to determine d as a function of z. For, writing equation (4) in the form

$$x-2yz+cz^2=0,$$

by equation (6) it becomes

$$y^2-2ycz+c^2z^2=0, \quad \text{or} \quad y=cz, \tag{7}$$

whence $$d=c^2z^2.$$

and equation (3) takes the form

$$y\left(\frac{x}{z}-y+cz\right) = c^2z^2,$$

or, finally,

$$\frac{y}{c}\left(\frac{x}{cz}-z\right)-\left(\frac{y}{c}-z\right)^2 = 0. \tag{8}$$

At a *point of diversion*, where $dz/dy = 0$, we have the equations

$$y^2 = cx,\ y = cz, \quad \text{whence} \quad x = cz^2,\ x = yz. \qquad (6, 7, 9, 10)$$

Further, when x is constant, $dz/dy = 0$ leads in a similar manner to equation (4) and the associated equations (6, 7, 9, 10). These last two results can easily be seen from the geometry alone of the figure.

Equation (8) is the equilibrium condition of the theoretical equation (2), with the substitution of z in place of a and 2 in place of n.

Equation (1)

$$\frac{dz}{dt} = \frac{y}{c}\left(\frac{x}{cz}-z\right)-\left(\frac{y}{c}-z\right)^2$$

has now been arrived at. To prove that it is correct, it must agree with experimental results for all values of y, x, z, and t. I shall show from certain of these that it does so.

If experiments be well planned, the constants c and z can be obtained with ease. The simplest mode of procedure is to erect an experiment on a line $x =$ constant, choosing a low value of x. The concentration of y, which gives the greatest degree of lysis, fulfils the requirements of equations (6), (7), (9), (10), for $y^2 = cx$ is the family curve of the points of diversion, and the tangent at the point of diversion is parallel to the y axis.

If a small series of this sort be put up, the tube which shows the first flash of lysis may be taken as an approximation, and a further series of tubes with concentrations differing only slightly from this may be put up forthwith. When the concentration of y which causes maximum lysis in the series has been determined, the equation $y^2 = cx$ evaluates c. The maximum degree of lysis having been evaluated colorimetrically, the equation $x = yz$ gives the value of z for that degree of lysis.

In the experiment which I have described, another method had to be adopted. As I have shown, the asymptote for a constant value of z is $x/z-y+cz = 0$, and its slope $dx/dy = z$.

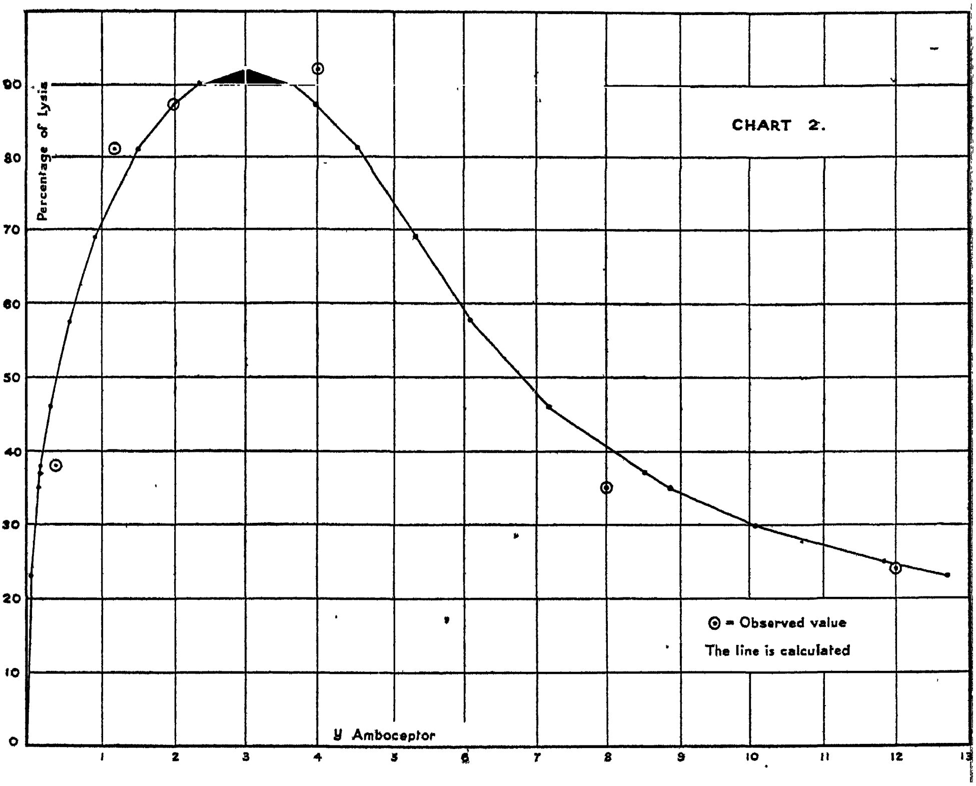
In the series (Chart 1) erected on the line $x = y$, complete lysis values are succeeded by incomplete in the upper half of the line. Thus, z for complete lysis is greater than 1. I chose its value at 1·25.

In the series erected on the line $x = 0{\cdot}5y$, partial lysis values are succeeded in the upper part of the line by a value "slight—partial." Thus, z for partial lysis is slightly greater than 0·5. I chose its value as 0·55.

The point of diversion for the curve of complete lysis appeared to lie near the point ($y = 15$, $x = 19$), from equation (6) $y^2 = cx$; thus, $c = 11{\cdot}8$. These approximations were subsequently modified to $c = 11{\cdot}37$, and

$$z \text{ for complete lysis} = 1{\cdot}256,$$
$$z \text{ for lowest partial lysis} = 0{\cdot}54.$$

II. y and z varying; x constant. $dz/dt = 0$. *Vide* Chart 2.—The following experiment is taken from Arrhenius ('Immuno Chemistry,' English Edition, p. 225), for his value $b = 6$, *i.e.*, $x = 0{\cdot}24$ per cent. I have expressed his units in percentages, *e.g.*, $y = 2$ means $a = 50$:—



Percentage lysis.	y used.	y calculated.
37	0·4	0·169
81	1·2	1·52
87	2·0	2·01
92	4·0	3·0
35	8·0	8·9
24	12·0	12·14

$c = 37{\cdot}5$, $z = 0{\cdot}08$ for 92 per cent. lysis.

The constants were obtained as follows:—

The summit of the observed curve (*vide* Chart 2) is shown as lying between 3 and 4. I chose $y = 3$ as the summit, and that the degree of lysis at this point was 92 per cent. The value of x is 0·24 throughout the whole curve. The summit conditions are given by equations (6) and (7).

By equation (6) $$y^2 = cx$$

we have $$c = 9/0{\cdot}24,$$

or $$c = 37{\cdot}5.$$

By equation (7) $$y = cz,$$

$$z = 3/37{\cdot}5.$$

Therefore $$z = 0{\cdot}08 \text{ for 92 per cent. lysis.}$$

A comparison of observed and calculated values in Chart 2 shows more clearly than the figures do that all the singularities of the curve are fulfilled.

III. x and z varying; amboceptor (y) constant. $dz/dt = 0$. Chart 3.—To find the constants in the (x, z) plane is much more difficult. A point of flexion may be found within the range of experiment. When little amboceptor is present it lies near the y axis. When much more amboceptor is present it is not seen; the curve is nearly flat; the point, as a matter of fact, lies beyond the line of complete lysis.

The curve thus occurs in widely different forms. For low values of y it is almost entirely convex upwards. For high values of y it is concave upwards, in reality nearly flat, and for intermediate values it assumes an $\int$ form. Some of these forms are shown in Chart 3.

The point of flexion can be found mathematically as follows. Re-arrange equation (8)

$$x + cz^2 = yz + c^2z^3y^{-1};$$

thus $$\frac{dz}{dx} = \frac{1}{y + 3c^2z^2y^{-1} - 2cz},$$

and $$\frac{d^2z}{dx^2} = \frac{2c - 6c^2zy^{-1}}{(y + 3c^2z^2y^{-1} - 2cz)^2}\,\frac{dz}{dx}.$$

When $$\frac{d^2z}{dx^2} = 0 \text{ (the point sought for),}$$

either $$\frac{dz}{dx} = 0, \text{ which is obviously not the case,}$$

or $$6c^2zy^{-1} = 2c, \quad i.e. \quad y = 3cz. \tag{11}$$

Substituting this value of y in (8), we find

$$x = \tfrac{7}{3}cz^2. \tag{12}$$

Consequently,

$$\begin{cases} y^2 = 3{\cdot}8571cx, & (13) \\ x = \frac{7}{9}yz. & (14) \end{cases}$$

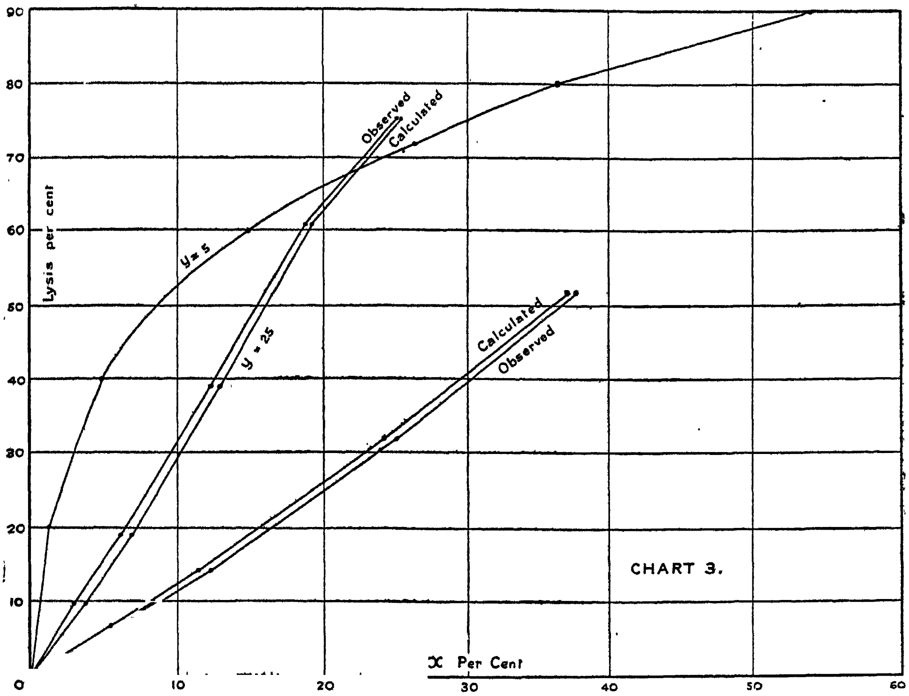


CHART 3.

The following experiment shows these facts very clearly. Normal human serum was made up in the following proportions:—

	Unheated serum.	Heated serum.	*I.e. y.*	*x.*
	parts.	parts.		
A	4	0	4	4
B	3	1	4	3
C	2	2	4	2
D	1	3	4	1
E	1	7	8	1

Tubes were put up in two series.

	c.c.
Series 1.—Serum mixture (A, B, C, D, or E)	1·00
0·85-per-cent. NaCl solution	0·95
Washed horse cells	0·05
Total	2·00

i.e. in this series amboceptor = 50 per cent.

		c.c.
Series 2.—Serum mixture (A, B, C, D, or E)	....	0·50
	0·85-per-cent. NaCl solution	1·45
	Washed horse cells	0·05
	Total	2·00

i.e. in this series amboceptor = 25 per cent.

The tubes were incubated at 37 C. for *two hours*, and kept on ice overnight. They were then estimated for lysis by colorimetry.

The results are as follows (*vide* Chart 3):—

		Observed lysis.	z.	x used.	x calculated.
Series 1. $y = 50$ ...	B	52·0	0·86	37·5	36·87
	C	32·0	$0{\cdot}5\dot{3}$	25·0	24·12
	D	14·6	$0{\cdot}24\dot{3}$	12·5	11·603
	E	6·8	$0{\cdot}11\dot{3}$	6·25	5·54
Series 2. $y = 25$ ...	A	80·4	1·34	25·0	25·16
	B	61·0	$1{\cdot}01\dot{6}$	18·75	19·284
	C	39·2	$0{\cdot}65\dot{3}$	12·5	13·17
	D	19·0	$0{\cdot}31\dot{6}$	6·25	7·02
	E	9·8	$0{\cdot}16\dot{3}$	3·125	3·83

Constants $c = 10$, $z = 0{\cdot}8\dot{3}$ for 50 per cent. lysis.

The constants were obtained as follows:—

The observed results having been charted it was observed that in the curve for ($y = 25$) there was a point of inflexion whose co-ordinates were approximately (Lysis = 50 per cent.) ($x = 15{\cdot}5$).

By equation (11) $y = 3cz$, thus $cz = 8{\cdot}\dot{3}$.

By equation (12) $x = \frac{7}{3}cz^2$, thus $z = 0{\cdot}797$.

Substituting this value in (11),

$$c = 8{\cdot}\dot{3}/0{\cdot}797 = 10{\cdot}45.$$

These approximations were subsequently modified to $c = 10$ and $z = 0{\cdot}8\dot{3}$ for 50 per cent. lysis.

Chart 3 shows more remarkably than the tabulated results do, how complete is the accordance between the theoretical and the observed forms of curve. (In the lowest curve in Chart 3, $y = 50$.)

The ∫ form, with its point of flexion, is shown for the value $y = 25$, and the preliminary concavity for the higher value $y = 50$. An experimental point of note is that for observed results to agree with the equation of equilibrium, it is absolutely necessary that the reaction should be allowed to continue for a sufficient period of time. One hour at 37° C. is insufficient; under such conditions the bulges in the ∫ form are much more developed, but the curve is not calculable by equation (8), as the system is not in equilibrium. I have found that incubation for two hours at 37° C., followed by 12 hours in the ice box, is sufficient for practical purposes.

The variables y, x, and z, have now been fully observed; and the surface obtained by the equation of equilibrium agrees in every detail and in all its singular properties with the form obtained from experiment.

IV. y and t varying. Complement absent ($x = 0$); effect z constant. This leads us into a different domain of serum reactions, viz., agglutination. Equation (1) loses its fraction dependent on concentration of complement, and becomes

$$\frac{dz}{dt} = -\left(\frac{y}{c} - z\right)^2. \tag{15}$$

On integration

$$-\frac{yt}{c} = \frac{z}{\frac{y}{c} - z} \quad \text{or} \quad yt = \frac{c^2 z}{cz - y}. \tag{16}$$

The appended table is taken from Arrhenius,* and I have added as a fourth column results calculated from the right hand of equation (16)—

y	t (minutes).	yt	$c^2z/(cz-y)$.
0·008	140	(1·12)	1·11
0·006	150	0·90	0·95
0·0055	165	0·91	0·912
0·005	180	0·90	0·888
0·004	210	0·84	0·83
0·0035	240	0·84	0·80
0·003	300	0·90	0·784
0·0022	420	0·92	0·75
0·002	480	0·96	0·74

$c = 0·66, \quad z = 0·03.$

These constants were obtained by solving simultaneous equations obtained by introducing observed values of y and t in equation (16).

Arrhenius considers that there is an experimental error in the first value of yt, due to the fact that the liquids were not immediately brought to a constant temperature, and considers yt to be constant (mean for last eight values = 0·90). I would suggest that the discrepancy lies in the later figures. Agglutination is measured by the eye. When the reaction is fast the flocculi are larger and are more easily observed. When the reaction is slow agglutination occurs in smaller, more discrete particles, and observed times would tend to be too long. (I have presumed that, in this experiment, the number of bacteria was sufficient to take up all the amboceptor. I shall discuss this in Section VIII.)

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* *Loc. cit.*, p. 104.

V. *The Relation between y and x which gives a Maximum Velocity.*—

$$\frac{dz}{dt} = \frac{y}{c}\left(\frac{x}{cz} - z\right) - \left(\frac{y}{c} - z\right)^2, \qquad \frac{d}{dy}\left(\frac{dz}{dt}\right) = \frac{x}{c^2z} + \frac{z}{c} - \frac{2y}{c^2},$$

when

$$\frac{d}{dy}\left(\frac{dz}{dt}\right) = 0$$

(*i.e.*, when increase of velocity per increment in y is zero), then

$$2y = x/z + cz,$$

and this equation is satisfied when

$$y = cz, \quad x = cz^2, \quad y^2 = cx, \quad x = zy,$$

i.e. at "points of diversion."

This conclusion is made use of in the method of finding the approximate value of c, as described at the end of Section I.

VI.—*When the Reaction is performed in Two Stages* (Supersensitisation phenomena).—Let y be $> cz$. The first part of the experiment follows the equation

$$\frac{dz}{dt} = -\left(\frac{y}{c} - z\right)^2.$$

When saturation has been accomplished,

$$\frac{dz}{dt} = 0, \qquad t = \infty, \qquad \frac{y}{c} = z.$$

If z for complete lysis was z_0 at the commencement of the reaction, it is after the first part of the experiment equal to $y/c \, . \, z_0$ (call this z_1).

If complement be now added, the reaction follows the equation

$$\frac{dz}{dt} = \frac{y}{c}\left(\frac{x}{cz_1} - z_1\right).$$

(The value y/c is retained as all the amboceptor has become attached.)

When equilibrium has been reached, $x = cz_1^2$.

Had the experiment been performed in the usual manner in the presence of both components, equilibrium would have been reached when

$$\left(\frac{y}{c} - z_0\right)^2 = \frac{y}{c}\left(\frac{x}{cz_0} - z_0\right).$$

That is to say the "point of diversion" would have been at

$$y = cz_0, \qquad x = cz_0^2.$$

Thus the value of x at the point of diversion in the divided experiment would be

$$x_1 = c\left(\frac{y}{c}z_0\right)^2;$$

in the simultaneous experiment

$$x_0 = cz_0^2.$$

The state of affairs is shown simply in the accompanying diagram :—

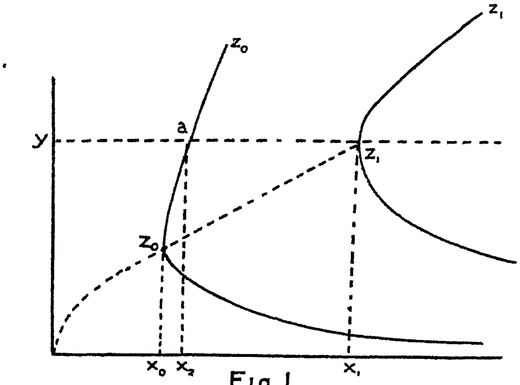

Fig I.

Let us suppose that the serum employed contained y of amboceptor and x_2 of complement. In an ordinary simultaneous experiment, complete lysis would occur at the point a, the curve for complete lysis being the line az_0.

If, however, the experiment is performed in two stages, the initial action of amboceptor y is to shift the line of complete lysis from z_0 to z_1. Consequently, the line z_0 will now be a line of partial lysis, the available quantity of complement x_2 being insufficient to lyse the supersensitised cells. If x_2 be increased to x_1, complete lysis will be attained. An advanced degree of this phenomenon is shown in the following experiment:—*

Inactive dog serum, *i.e.* y.	A. Consecutive reaction.	B. Simultaneous reaction.
c.c. 1·0	$z = 0$	z = complete lysis.
0·5	„	„ „
0·35	„	„ „
0·25	„	„ „
0·15	„	= nearly complete lysis.
0·0	„	= 0.

$x = 0·5$ c.c. guinea-pig serum. Cells = 1 c.c. of a 5-per-cent. suspension from guinea-pig.

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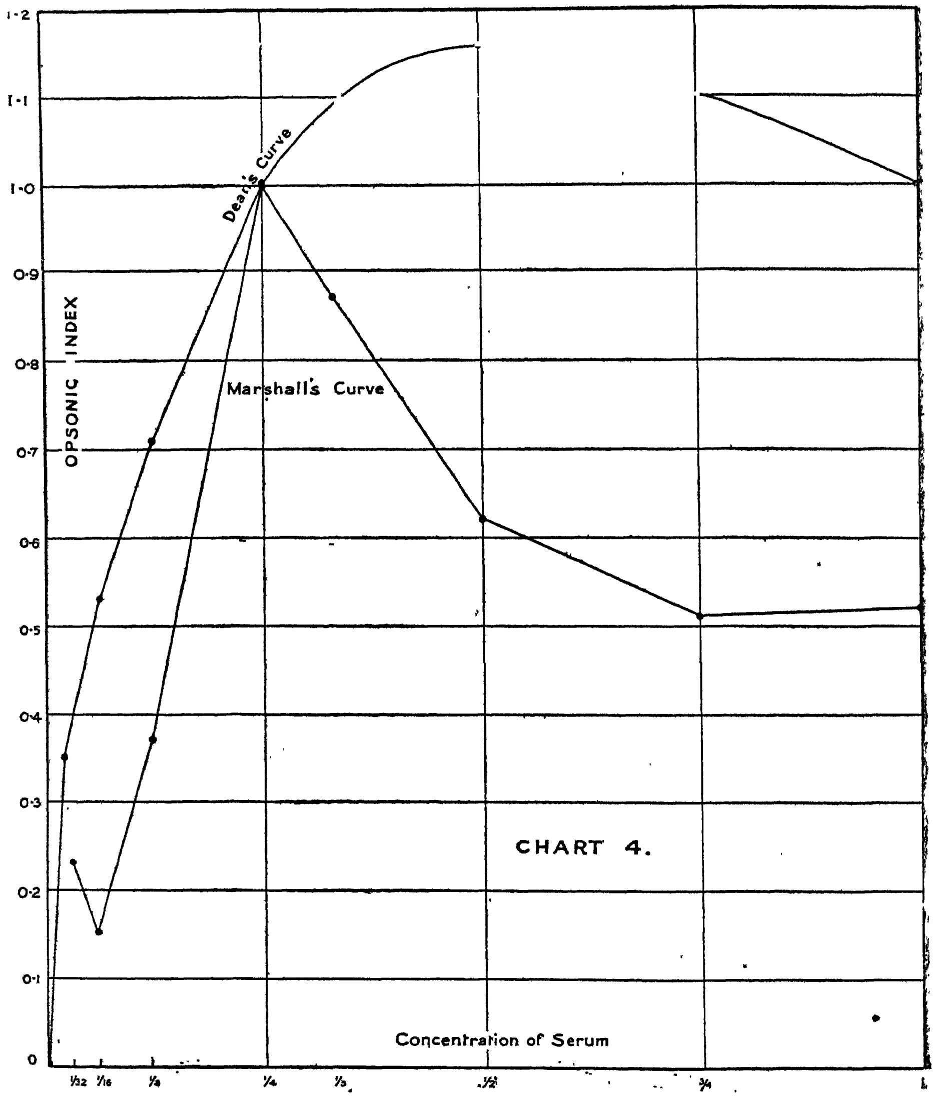
* Ehrlich, 'Studies in Immunity,' English edition, p. 211.

VII. *Opsonins and Stimulins.*—Papers by Dean* and Marshall† contain many interesting observations on the effect of dilution in opsonin and stimulin experiments.

Chart 4 shows two curves: one from Dean, showing the effect of dilution on the opsonic index in a normal serum; one from Marshall, in the serum of a horse, "Rich," which had been immunised against staphylococcus, the treatment covering a period of about two years.



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* 'Roy. Soc. Proc.,' B, vol. 79.

† 'Journ. of Path. and Bact.,' 1908, vol. 12.

Comparison of these curves (especially that of Dean) with Chart 2 shows a marked similarity. Stated shortly, the characteristics of opsonin and stimulin reactions are as follows:—

(1) *An unheated normal* serum induces phagocytosis of organisms by leucocytes.

(2) *A heated normal* serum induces practically no phagocytosis.

(3) *An unheated immune* serum induces strong phagocytosis.

(4) *A heated immune* serum induces marked phagocytosis.

The phenomenon of phagocytosis consists of two stages: firstly, approximation of the organism to the leucocyte, probably by some amboceptoral action, possibly by chance collision; and, secondly, inclusion of the organism by the leucocyte. The leucocyte is undoubtedly a source of complement, so there may be present a zone of complement in or about it.

Let us return to the comparison between the curves on Charts 2 and 4, presuming for the moment that complement takes part in the reaction.

If complement acted proportionally to its concentration in the serum, then the experiment would appear on a plane erected on the line $y = x$.

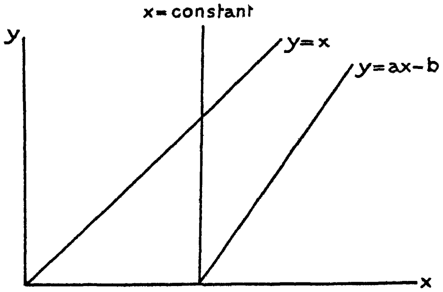

Fig 2.

Rearranging equation (8), putting $y = x$, and extracting roots, we have

$$y = \frac{cz^2 \pm cz\sqrt{z(4-3z)}}{2(z-1)}.$$

y can have only one positive value when $z < 1$;

y has equal roots (*i.e.*, the summit of the curve)

when $3z = 4$, or $z = 1\cdot\dot{3}$.

Thus if lysis at the summit = 100 per cent., it cannot fall below 75 per cent. in the plane $(z, y = x)$.

Marshall's curve, however, falls to 50 per cent. at least, whilst Dean's falls (within the limits of experiment) to 86 per cent.

Let us now return to the characteristics (1 to 4) above.

The fact that unheated sera act better than heated sera shows that the complement of the serum is called into play.

That a heated normal serum induces practically no phagocytosis, whereas a heated immune serum may induce a marked reaction, would suggest that the amboceptor (which is present in large quantity in the latter) acts as a stimulant on the leucocyte to secrete complement.

From this point of view Marshall's curve would lie in the plane $(z, y = ax-b)$. Dean's curve, on the other hand, which is that of a normal serum (which when heated would yield practically no phagocytosis), would lie either in the plane $(z, y = ax)$, or $(z, y = ax-b)$ where b is a very small quantity. I have calculated this curve, both in planes $(z, y = ax)$, in which case the observed value of z with full concentration lies too low, and in the plane $(z, x = b)$, in which case the observed value is too high.

Thus, to summarise, amboceptor and complement both take part in the reaction. Complement is supplied by the serum (if this source be available) or from the leucocyte, by virtue of stimulation of the cell by the amboceptor—a stimulation to secrete complement, which acts locally.

The curves of Dean and Marshall (Chart 4) are merely further exhibitions of the phenomenon of complement "diversion." (Marshall also gives curves of heated immune sera experiments which show the diversion phenomenon.)

The following points may be of use in calculating curves and finding the values of constants in the plane $(z, y = ax)$. When $y = ax$, equation (8) assumes the form

$$y = c\left(\frac{az^2 \pm z\sqrt{az(4-3az)}}{2(az-1)}\right). \tag{17}$$

z has a maximum value when $4 = 3az$, or $z = 1\cdot\dot{3}/a$, and consequently $y = 2cz = \frac{8}{3}\frac{c}{a}$. z has only one positive value when $z < 1/a$. Thus $z = 1/a$ is the asymptote.

Now by the equation (10) $y = x/z$ at the "point of diversion," and consequently in the plane $(z, y = ax)$ a point of diversion occurs when $z = 1/a$. Thus if the value of z on the asymptote is known, we know its value at the "point of diversion." In whole serum experiments $y = x$, consequently $x = 1$ at the point of diversion and on the asymptote.

VIII. *The values c and z (Immunity).*—(*a*) c is a constant inversely proportional to the amount of y present in the undiluted serum.

(*b*) z is a complex factor. It is, from a chemical point of view, the amounts of x and y which have been transformed. It is in general the *effect*. It includes, degree of effect, resistance to effect, concentration of stuff acted upon (in the special case of hæmolysis, the number of cells acted upon). In equation (1) it was used to denote the ratio x/y (see equation 10).

Thus $$z = x/y\mathrm{E}f(n)r, \tag{18}$$

where E = effect,

$f(n)$ = a function of the number of cells, or the concentration of stuff acted upon,

r = internal resistance (*e.g.*, of the cell),

R = any external resistance, and a function of the temperature.

Since y/c and x/cz measure the total quantities of amboceptor and complement in a serum, the determination of c and z will afford absolute values for these two components; $1/c$ will be the measure of units of amboceptor, and $1/cz$ the measure of units of complement. An increase in z will correspond to a decrease or deterioration of complement, and an increase in c to a decrease in amboceptor.

In immunisation, however, amboceptor is supposed to increase, and complement to remain more or less constant. Thus in immunisation there would be a decrease in c and a corresponding increase in z. It is on this basis that indices of immunity, obtained from experiments in which both amboceptor and complement take part, must rest. The present system of bacteriolytic and opsonic indices, based on the observation of a single dilution of the serum to be investigated, is and must be liable to error. In the case of the horse "Rich" the opsonic index for the whole serum had fallen, after two years' treatment with staphylococcus, although I believe that the content in amboceptor had actually increased. I would point out that an animal such as this one, even although its opsonic (or other compound) index had decreased, would be more potent as a source of anti-staphylococcic serum than it was when its index was high. Such an animal should not be discarded without careful testing.

This fact is also of extreme importance with regard to treatment of patients by immunising. For instance, the opsonic index obtained by whole serum evaluation would fall off in the later stages of a protracted course of treatment, even although the serum had gained in amboceptoral content. I do not think that this would be a dangerous state of affairs from the patient's point of view, as possibly he may possess resistive power in a potential state, but the fact is worthy of note and of research.

With regard to the value $f(n)$. Throughout this paper, and in my experiments, I have taken n to be sufficiently great to absorb all the amboceptor used. But other conditions may prevail. For instance, in opsonic experiments there is a limit in the size of the cell, which would present itself, if only a very small number of them was present. In agglutination experiments, if the organisms were few in number, a portion of y would remain unabsorbed.

IX. *Toxins and Antitoxins.*—Ehrlich has postulated that the toxin molecule has two receptors, a toxophorous, which is comparatively thermolabile, and a haptophorous, which is thermostable. Arrhenius has stated that these receptors may be considered to belong to two different molecules. I shall go a step further, and by imitating the chief characters of toxins, and their neutralisation by antitoxins, show that these two factors are probably amboceptor and complement, and that the antitoxin is an anti-amboceptor:—

(*a*) A primary characteristic of toxins is that different samples may agree in their minimal lethal dose (M.L.D.), whilst they disagree in the amount of antitoxin necessary for their neutralisation.

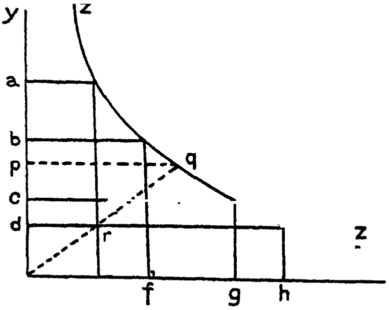

Fig 3.

Fig. 3 is a form of chart obtained as on Chart 1, z = effect (in Chart 1, z signified complete lysis, *i.e.* death of the cell. It may now be considered as a "lethal" line).

Toxins consisting of—

$$ay+ex, \qquad cy+gx,$$
$$by+fx, \qquad dy+hx,$$

all have the same lethal effect, but the quantity of an anti-amboceptor requisite for neutralisation would be a, b, c, d, equivalents respectively.

(*b*) A second characteristic is that a toxin which has deteriorated in lethal effect still requires the same amount of antitoxin to neutralise it. This is obviously a corollary of (*a*).

For instance, let a toxin $dy+hx$ deteriorate to $dy+ex$; d equivalents of anti-amboceptor will be required for neutralisation of both the fresh and the deteriorated states, but the lethal effect will have been much diminished. As amboceptor has been considered to remain constant during deterioration, the lethal dose may be measured in terms of amboceptor. In the fresh state the dose is measured by od, and in the deteriorated by op (fig. 3).

(*c*) A third characteristic is in the curious curve which toxicity (as ordinate) assumes, as increasing quantities of antitoxin (as abscissa) are added. The curve is an ʃ written backwards. At first it is almost flat (ascribed by Ehrlich to prototoxoid), then it descends more sharply, and finally it becomes again flatter. Arrhenius has shown that the flat finish can be explained on the principle of mass action equilibria between toxin and antitoxin, but the flat commencement cannot thus be explained.

Toxicity is inversely proportional to the minimal lethal dose, and is to be distinguished from z, which means degree of effect.

Minimal lethal dose can be shown in fig. (3) as follows: Let 1 c.c. of a toxin contain $dy+ex$. We can measure this by or. Thus $or = 1$ c.c. Now, to cause death, or must be produced to oq, the lethal line, that is to say, the minimal lethal dose is oq/or c.c., and the toxicity $= 1/\text{M.L.D.} = or/oq$.

Now suppose we have a toxin containing $ay+ex$ in 1 c.c., and that, after acting on it with anti-amboceptor, ay is reduced to dy.

The lethal dose of $ay+ex = 1$ c.c. (*vide* fig. 3),

but the lethal dose of $dy+ex = (oq/or)$ c.c.

The toxicity is or/oq.

If we assume, as a simple case, that ay is neutralised by a units of antitoxin, then the curve of toxicity is flat at first, and becomes more and more steep as more antitoxin is added. But if we assume with Arrhenius that complete neutralisation never occurs, then this curve is drawn out in its final part, and never reaches the abscissa.

Thus the prototoxoid phenomenon will appear in certain cases, and I would add that it is possible that the curve might even commence with a rise, *i.e.* a toxin might be found which became apparently more toxic on addition of a small quantity of antitoxin. I am not aware that this has been observed.

These three primary characteristics of toxin and antitoxin have thus been shown to be fully described by the equilibrium relation of equation (1) taken in conjunction with Arrhenius's views; the haptophorous group being represented by amboceptor, the toxophorous group by complement, and antitoxin by anti-amboceptor.

Summary.

1. Amboceptor and complement are opposed in their action on the cell, with the proviso that the former acts as a catalyst to the latter.

2. Complement action is lytic; amboceptoral action is primarily polymerising, or, as the case may be, agglutinative; and, secondarily, catalytic to complement.

3. The relation of these substances is expressed by the law of mass action, in the form

$$\frac{dz}{dt} = \frac{y}{c}\left(\frac{x}{cz} - z\right) - \left(\frac{y}{c} - z\right)^2.$$

4. When the substance acted upon is in sufficient quantity, this expression describes all serum reactions, viz., hæmolysis, bacteriolysis, opsonin and stimulin reactions, agglutination, precipitation and toxin action.

5. Toxins are compound and consist of amboceptor and complement.

[*Note.*—The idea that amboceptor and complement were opposed in their actions has been held by Captain W. F. Harvey and myself for many years. We joined in many experiments to prove our idea. It was only recently that I determined to investigate diversion phenomena from a mathematical point of view. I did so, and found, at the end of my work, that I had again to have recourse to this same view of opposed action, a result which I had not foreseen. Captain Harvey and I were in the habit of calling amboceptors *polymerins* and complement *lysin*, and I know of no better names to describe their properties. Amboceptoral action is always, when acting alone, a polymerising one, whereas agglutination may, or may not, occur in the course of polymerisation. Agglutination is a molar, whereas polymerisation is a molecular phenomenon. Indeed, as has been shown by Muir, reduction of polymerisation by complement may cause agglutination.]

I have also to express my thanks to Major Cornwall and Dr. Kesava Pai, the Director and Assistant Surgeon of this laboratory—the Pasteur Institute of Southern India.

Further Researches on the Development of Trypanosoma gambiense *in* Glossina palpalis.

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(Received February 15,—Read March 16, 1911.)

The object of these experiments was to try to discover if there is any definite cycle of development of the trypanosome of Sleeping Sickness in the tsetse fly, *Glossina palpalis*, and if the late or renewed infectivity of the fly coincides with any phase in this development.

The mode of experimentation was to feed a cageful of *laboratory-bred* tsetse flies on an animal whose blood contained numerous trypanosomes, and at the end of various times to kill the flies and examine their intestinal contents. This was done for periods of one day, two days, three days, and so on, up to 56 days. The microscopical examination of preparations made from the intestinal contents on the various days gave information as to the number and appearance of the trypanosomes.

After the infective feed or feeds the flies were fed every day on a healthy animal, so that by the appearance of trypanosomes in the animal's blood the day on which one or more of the flies became infective could be arrived at.

METHOD USED IN THE EXAMINATION OF THE FLIES.

The flies were killed by exposing them to the vapour of chloroform. After being killed the proboscis and pharynx were removed and examined under a cover-glass with the high and low powers. The terminal segment of the abdomen of the fly was then snipped off, and the whole abdominal viscera gently pressed out. This was moistened with a little normal saline solution, and the gut unravelled without rupturing. The proventriculus and crop were often pulled out intact with the gut. The whole thoracic and abdominal tract could then be laid out in line and examined under a low power.

In taking out the gut it was generally possible to draw out with it the abdominal portion of the salivary glands, which could then be separated without contamination from accidental rupture of the gut. If the salivary glands or proventriculus remained behind they were dissected out after removal of the gut. In every case these organs were thoroughly washed in three changes of normal saline solution, in order to minimise the chance of their being contaminated by accidental rupture of the intestines.

The stained specimens were examined day after day, and coloured drawings, at a magnification of 2000 diameters, made of all the different forms met with. The drawings of the trypanosomes found in the proboscis, proventriculus, fore-gut, mid-gut, hind-gut, proctodæum, and salivary glands, were kept separate, so that a series of drawings of trypanosomes taken from any one part, from the first day of infection to the 56th day, could be compared.

By arranging these drawings on a wall the horizontal layers would represent the contents of, say, the fore-gut from one day onwards, while the vertical rows would represent the trypanosomes found in the whole length of a fly for one day. More than six hundred drawings were made in this way, so that it seems impossible that any important form can have been left out.

GENERAL CONSIDERATIONS REGARDING THE DEVELOPMENT OF THE TRYPANOSOMES IN THE FLY.

Let us now take a general view of the types of trypanosomes found in the various parts. It is evident that very few of the six hundred drawings can be reproduced; a few types, taken here and there, must suffice. For the first three or four days trypanosomes are found in all the flies, but at the end of six or seven the trypanosomes have disappeared out of many of them. That is to say, it is only in a certain percentage that further development takes place. In one series this was 8 per cent. In 92 per cent., then, of flies which imbibe infected blood, the trypanosomes simply degenerate and die out within the first few days. In 8 per cent., on the other hand, the trypanosomes find conditions more favourable for development, and increase to a marvellous extent, filling the whole of the fore-gut, mid-gut, and hind-gut with countless swarms of trypanosomes.

How long this development continues is unknown. It is considered probable that it continues for the remainder of the fly's life, and this has been proved by experiment up to 96 days.

What the conditions in the intestine of the fly are, which render this development possible, are also unknown. It was thought that it might depend on the reaction of the intestinal fluids. This is, however, found on examination to be faintly acid in all flies, whether development has taken place or not. The presence of bacteria also seems to have no connection with the phenomenon. Sex, moreover, has no influence; development occurs in as many males as females.

TYPES OF TRYPANOSOMA GAMBIENSE FOUND IN THE ALIMENTARY CANAL.

It would serve no good purpose to describe separately, day by day, the types found in the various parts of the alimentary canal, as they run into each other in such a way that any classification of them seems impossible.

The following table represents, approximately, the numbers found in the different parts of the alimentary canal at various times after infection. The — sign means that an examination was made and nothing found. ± means few. +, many. + +, very many. + + +, swarming. If no sign, then no examination has been made. (See p. 516.)

The Proboscis.—In our experience *Trypanosoma gambiense* is never found in the proboscis of *Glossina palpalis*, except immediately after an infected feed, when for a short time blood containing trypanosomes may be seen in the lumen of the proboscis. This is very different from what obtains in an infection by *Trypanosoma vivax*, in which case the proboscis is alone infected.

Proventriculus.—As seen from the above table, this part of the alimentary canal is sometimes found empty when the remainder of the gut is swarming.

Fore-, Mid-, and Hind-gut.—It is here that the greatest development of the trypanosomes is found. Among the extraordinary numbers and diversity of type it is difficult or impossible to find one's way. Generally speaking, the trypanosomes found during the first few days are merely degenerating blood forms. After this there appears a type of trypanosome which remains dominant throughout the whole developmental period. This is a long, moderately broad form, the protoplasm staining well, without granules or vacuoles, having an oval compact nucleus situated in the centre of the body, a small round micronucleus lying at some distance from the elongated snout-like posterior extremity, the undulating membrane narrow and simple, and the flagellum proceeding little, if anything, beyond the protoplasm of the cell. The flagellum also appears very frequently to arise from a pink-coloured body situated near the micronucleus, an appearance never seen in the normal blood trypanosomes.

This seems to be the healthy normal developing type in the intestine of the fly. It is seen in all parts of the intestine and at all times. It forms masses of innumerable individuals alike in size and shape. When a fresh supply of blood is taken in by the fly this type can be imagined to multiply with extraordinary rapidity. When the blood supply runs low then this type can also be imagined as degenerating and disappearing just as rapidly. The host of diverse forms which thus arises beggars description. Some are round or oval in shape, 3 or 4 microns in diameter, with or without a flagellum. From this simple form all shapes and sizes can be seen up to the huge shapeless mass of protoplasm, multi-nucleated and multi-flagellated.

Table I.—Number of Trypanosomes found in the Various Parts of the Alimentary Canal and Salivary Glands.

Time, days.	Pro-boscis.	Proventri-culus.	Crop.	General smear of gut.	Fore-gut.	Mid-gut.	Hind-gut.	Procto-dæum.	Salivary glands.
1	—	—				+ +			—
1	—	—				+	+		—
2	—				+	+	+ +		—
3	—					+ +	+ +		
4	—				+	+	+	—	—
5	—	—	—		—	—	—		—
6	—	—	—	+	+	+			—
7	—	—	—	+					—
8	—	+			+	+ +	+ +		—
8	—				±	±	±		
9	—	—			±	+ +		—	—
9	—				+	+	+		
10	—				+	+ +	+ +		
10	—					+			
11	—	±	—	+ +	—	±	±		—
11	—	±	—	+ +	+ +	±			—
11	—	+ +			+ +	+ +	+ +		—
14	—	+ + +	—		+ +	+ +		—	—
14	—	+ +	—		+ +	+ +	+ +		—
15	—	—			+	+			—
17	—	—	—		±	+ +	+ +		—
17	—	+ +		—	+ +	+ +			—
18	—	—			+ +	+	+ +		—
18	—					+ +	+ +		—
20	—	+ +			+ +	+ +	+ +	±	—
21	—			+ + +					
22	—			+ +					
23	—				+	+	+		
24	—				+ + +	+ + +	+ + +		
25	—	—	—		+	+ + +	+ +		+
28	—			+ +		+ +			+
30	—	+			+ +	+ +			+
30	—	+			+ +	+ +	±		—
31	—	+	+		+ + +	+ +			—
31	—			+					
34	—			+ +	+ +	+ + +	+ + +	—	—
35	—	—			+	+ +	+ +		—
36	—			+ + +	+ + +	+ + +	+		+
36	—	—		+ + +	+ +	+ + +			—
36	—	+ +		+ + +					
37	—			+ +					
40	—			+ +					
40	—			+ +					
42	—	+ +		+	+ +				+
43	—			+ +					+ +
44	—	+			+ +	+ + +			+
44	—				+	+ +	+ +		+
44	—	+			+	+	+		+
46	—	+			+ + +	+ + +	+ + +		+ +
49	—	—	—		+	±			—
51	—		—		+	+	+ +		—
53	—	—			±	±			—
53	—	—			+	+ +			+
53	—		—			±			—
56	—			+ +					+

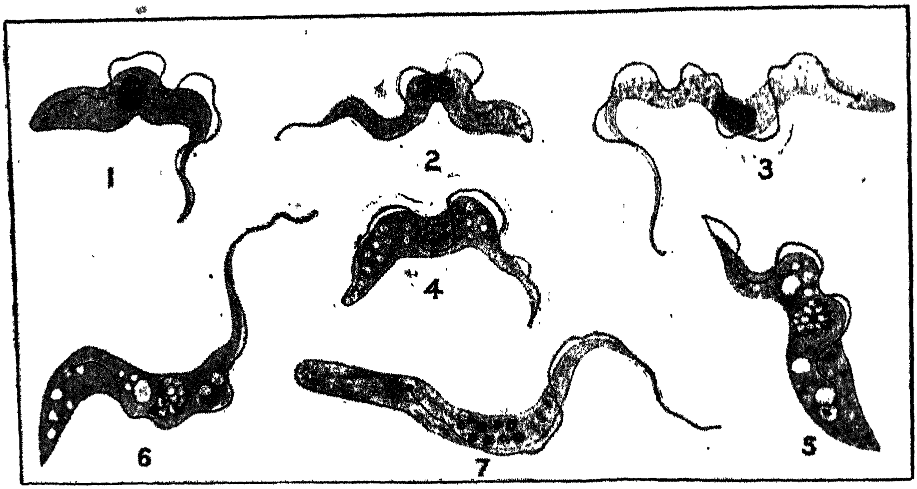
In our experience, the long narrow forms, described by some observers as "male" forms, are exceedingly rare, and it seems impossible to ascribe to to them any very important *rôle* in the process of development. Crithidia-like forms are also exceedingly rare—that is, forms with the micronucleus close to or anterior to the nucleus.

Types of Trypanosoma gambiense *found in the Salivary Glands.*—On referring to Table I it will be seen that trypanosomes did not appear in these glands until the twenty-fifth day, but that after this time they were usually present. In our opinion, this invasion of the salivary glands is of the greatest importance in the history of the development of *Trypanosoma gambiense* in *Glossina palpalis.* Here it may be parenthetically remarked that, because one kind of development takes place with one species of trypanosome and one species of tsetse fly, it by no means follows that the same thing will occur either with another species of trypanosome or another species of fly. Each combination must be worked out separately and nothing left to analogy. *Trypanosoma vivax* and *Glossina palpalis* afford a striking example of this.

In the development of *Trypanosoma gambiense* in *Glossina palpalis* one circumstance, which we think of prime importance, emerges, and that is, that in the salivary glands, and here alone, the trypanosomes are found to revert to the normal blood-type. It must not be imagined, however, that the salivary glands show no other forms but this blood-type. On the contrary, there are many other forms seen; but here only are found trypanosomes apparently identical with the short and stumpy forms found in the blood. What causes or leads up to this reversion to the blood-type in the salivary glands is quite unknown, but, as will be seen later, the *Glossina palpalis* does not become infective by biting until this invasion of the salivary glands takes place.

How the trypanosomes find their way into the salivary glands is also quite unknown. It seems highly improbable that they pass from the alimentary canal by way of the salivary duct, and as they are never found in the body-cavity, it is also difficult to see how they can make their way directly from the intestine to the abdominal portion of the salivary glands.

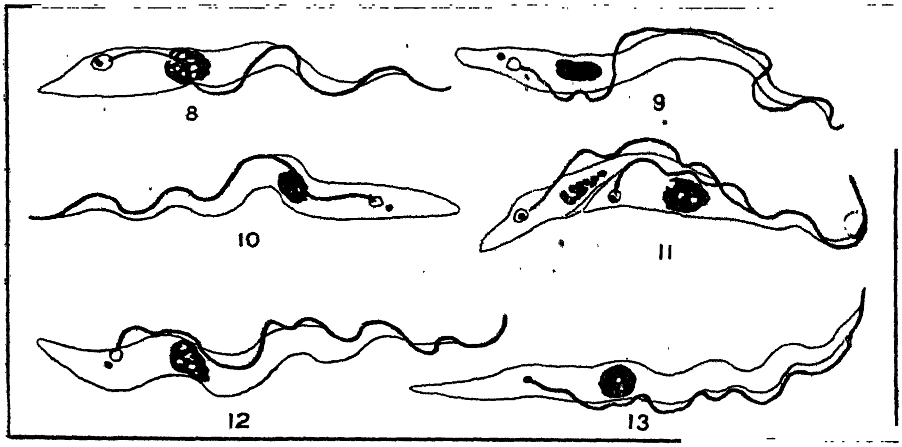
ILLUSTRATIONS OF VARIOUS MODIFICATIONS IN SHAPE OF TRYPANOSOMA GAMBIENSE IN GLOSSINA PALPALIS.



× 2000.

FIGS. 1—3.—Normal blood parasites (*Trypanosoma gambiense*).
FIG. 4.—24 hours after ingestion by the fly.
FIGS. 5 and 6.—48 hours after ingestion by the fly.
FIG. 7.—96 hours after ingestion by the fly.

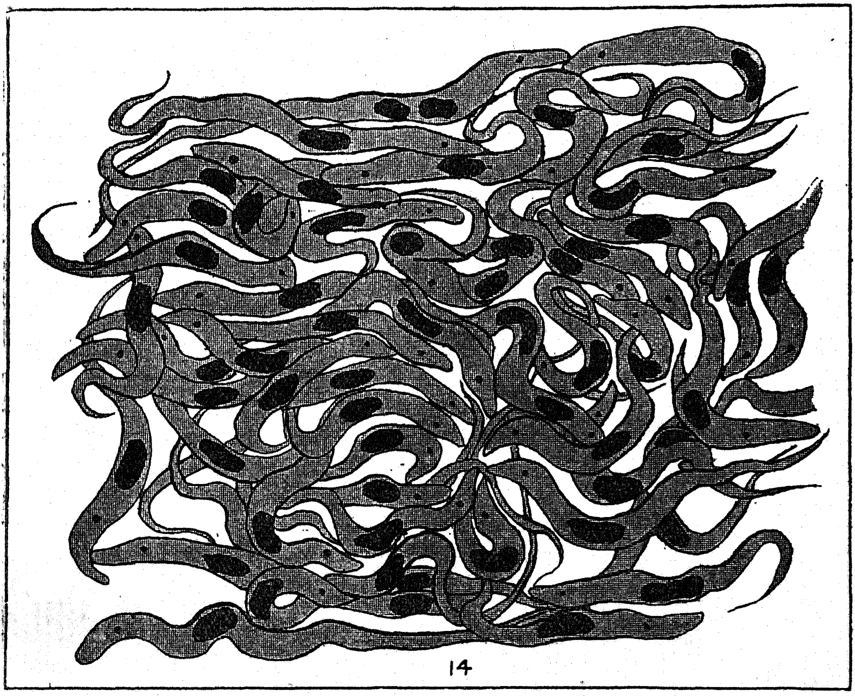
Figs. 1—7 represent the trypanosomes as they appear in the intestine of *Glossina palpalis* during the first few days. Figs. 1—3 are ordinary blood forms, as seen immediately after the fly has fed, and before any change has taken place. Figs. 4—7 represent the process of degeneration which takes place during the first four days. The body swells up, the nucleus breaks up, and the cytoplasm becomes vacuolated.



× 2000.

FIG. 8.—*Trypanosoma gambiense* from fore-gut, 8 days after infected feed.
FIG. 9.—*T. gambiense* from proventriculus, 14 days after infected feed.
FIG. 10.—*T. gambiense* from fore-gut, 18 days after infected feed.
FIG. 11.—*T. gambiense* from mid-gut, 25 days after infected feed.
FIG. 12.—*T. gambiense* from mid-gut, 34 days after infected feed.
FIG. 13.—*T. gambiense* from fore-gut, 44 days after infected feed.

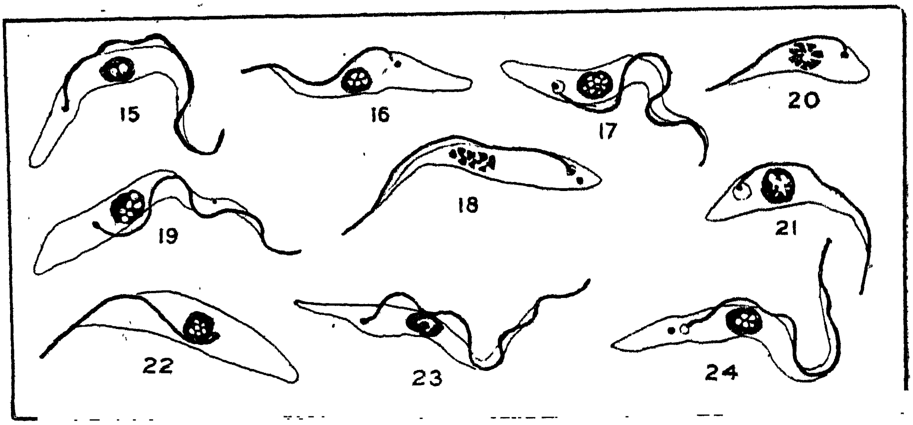
Figs. 8—13 represent what we consider to be the normal reproductive or developing type found throughout the intestine during the whole period of development. It is to be noted that this form is longer and broader than the normal broad form; the protoplasm is clear, and stains readily and evenly, and this cell looks normal. The nucleus is compact and situated nearer the posterior extremity than the anterior. The micronucleus is small and round, lying at some distance from the elongated posterior extremity. Many dividing forms of this type can be seen. In our opinion, this is the common multiplying form, and from it arises an infinite variety of degenerating forms.



× 2000.

FIG. 14.—Part of a mass of *Trypanosoma gambiense* from the mid-gut.

This figure is given in order to try to convey some idea of the enormous proliferation of trypanosomes which takes place in the intestine of *Glossina palpalis*. Throughout the whole length of the alimentary canal, from the proventriculus to the proctodæum, this condition is frequently seen, and in the living condition presents an extraordinary spectacle. Looked at through the wall of the intestine the matted masses are seen in active movement, swaying about and wriggling in every direction. They do not appear to be attached to the wall of the intestine, but move about freely, and when the intestinal wall is burst pour out in countless numbers. The trypanosomes evidently belong to the type figured in the preceding sketch, called the normal reproductive type.



× 2000.

Fig. 15.—*Trypanosoma gambiense* from hind-gut, 8 days after infected feed.
Figs. 16, 17, and 18.—*T. gambiense* from mid-gut, 14, 20, and 30 days after infected feed.
Fig. 19.—*T. gambiense* from hind-gut, 34 days after infected feed.
Figs. 20 and 21.—*T. gambiense* from proventriculus, 36 days after infected feed.
Fig. 22.—*T. gambiense* from hind-gut, 44 days after infected feed.
Figs. 23 and 24.—*T. gambiense* from mid-gut, 46 and 53 days after infected feed.

Figs. 15—24 represent smaller forms which are fairly common and occur throughout the intestine and at all times. The examples given above are taken from the eighth day to the fifty-third day. They are called by us "small developmental forms," since they resemble the larger in having clear protoplasm and a compact nucleus. Dividing forms are often seen.

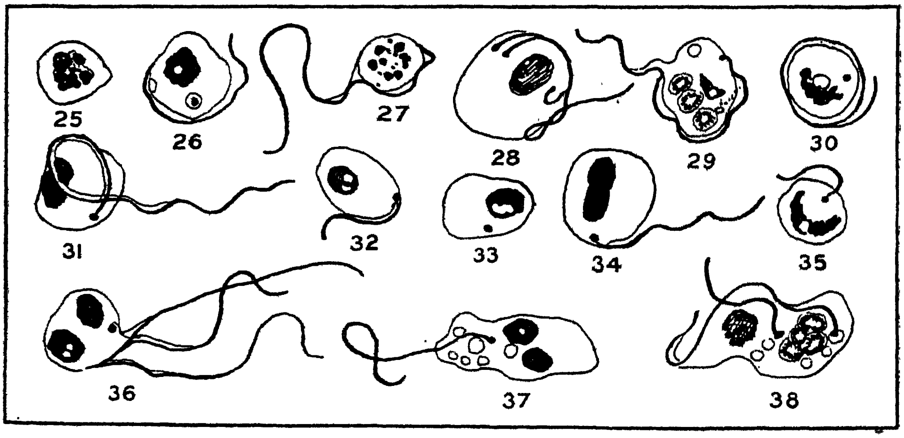


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Fig. 25.—*Trypanosoma gambiense* from mid-gut, 8 days after infected feed.
Fig. 26.—*T. gambiense* from fore-gut, 11 days after infected feed.
Figs. 27 and 28.—*T. gambiense* from mid-gut, 16 and 17 days after infected feed.
Figs. 29, 30, and 31.—*T. gambiense* from fore-gut, 17 and 18 days after infected feed.
Fig. 32.—*T. gambiense* from hind-gut, 20 days after infected feed.
Figs. 33 and 34.—*T. gambiense* from fore-gut, 24 days after infected feed.
Figs. 35, 36, and 37.—*T. gambiense* from mid-gut, 24, 44, and 46 days after infected feed.
Fig. 38.—*T. gambiense* from fore-gut, 46 days after infected feed.

Figs. 25—38 represent round and irregularly-shaped forms of the parasite, taken from the eighth to the forty-sixth day of development. It is impossible to say what is exactly the origin of these forms—whether by the segmentation of large masses, or simply by the division and subdivision of irregular forms. Some of the examples figured are evidently dividing, as they show several nuclei and flagella. Whether the round aflagellar forms correspond to the so-called "latent" forms of various writers, it is impossible to say. Those possessing flagella were active during life. In our opinion, they may be looked upon as part of the degenerative processes which are constantly taking place in the intestine of the fly.

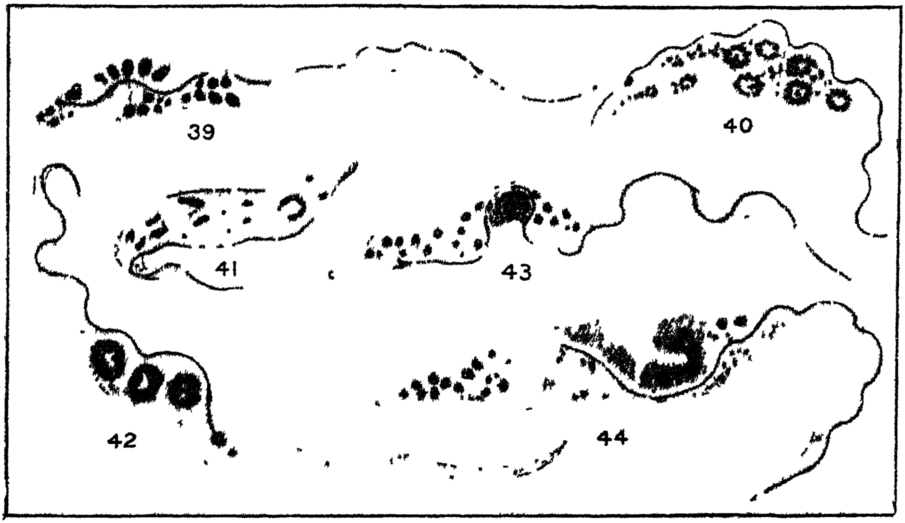

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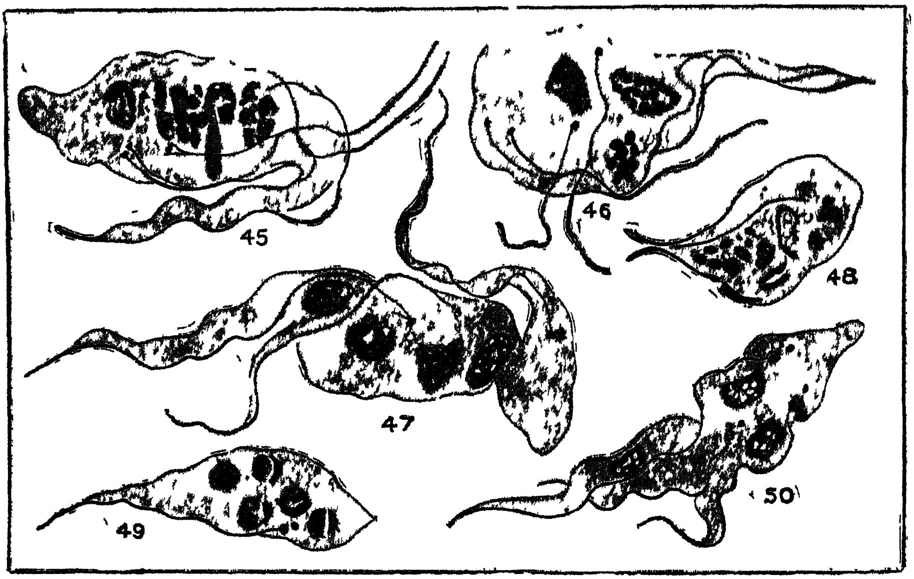
Fig. 39.—*Trypanosoma gambiense* from hind-gut, 10 days after infected feed.
Fig. 40.—*T. gambiense* from fore-gut, 17 days after infected feed.
Fig. 41.—*T. gambiense* from fore-gut, 17 days after infected feed.
Fig. 42.—*T. gambiense* from hind-gut, 34 days after infected feed.
Fig. 43.—*T. gambiense* from mid-gut, 34 days after infected feed.
Fig. 44.—*T. gambiense* from hind-gut, 46 days after infected feed.

Figs. 39—44 represent what appear to us to be degenerative forms of the "normal reproductive type." They are of all sizes and shapes, and the cell-contents are scattered over with broken-up nuclei, or at least granules of some stainable substance. Figs. 39, 43, and 44 are also vacuolated.



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Figs. 45, 46, and 47.—*Trypanosoma gambiense* from hind-gut, 10 days after infected feed.
Fig. 48.—*T. gambiense* from fore-gut, 17 days after infected feed.
Fig. 49.—*T. gambiense* from proventriculus, 30 days after infected feed.
Fig. 50.—*T. gambiense* from mid-gut, 46 days after infected feed.

Figs. 45—50 represent some of the more exaggerated types of degenerative forms. As will be seen from the drawings, they are huge, mis-shaped masses of protoplasm, multinucleated and, as a rule, multi-flagellated.

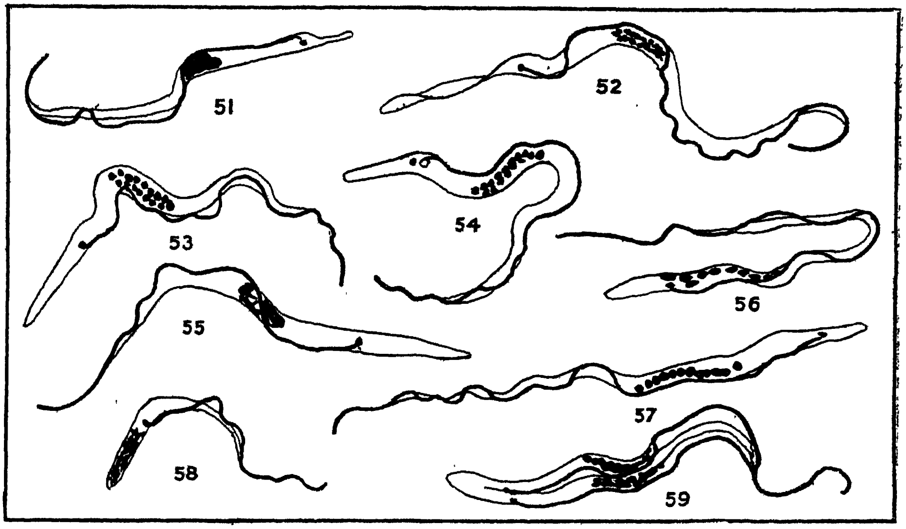

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Fig. 51.—Slender type of *Trypanosoma gambiense* from proventriculus, 17 days after infected feed.

Fig. 52.—Slender type of *T. gambiense* from mid-gut, 17 days after infected feed.

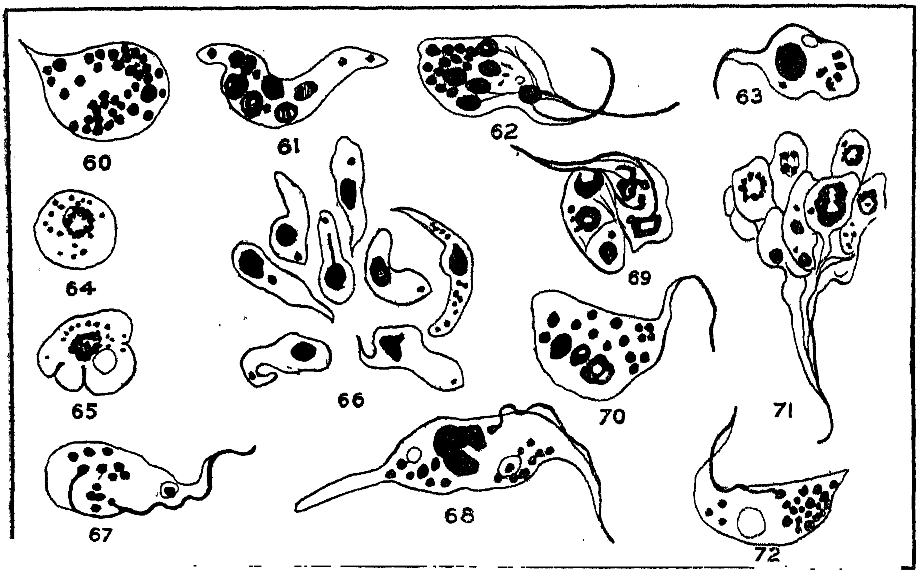
Fig. 53.—Slender type of *T. gambiense* from hind-gut, 20 days after infected feed.

Figs. 54 and 55.—Slender types of *T. gambiense* from fore-gut, 24 and 30 days after infected feed.

Figs. 56 and 57.—Slender types of *T. gambiense* from proventriculus, 44 days after infected feed.

Figs. 58 and 59.—Slender types of *T. gambiense* from fore-gut, 46 days after infected feed.

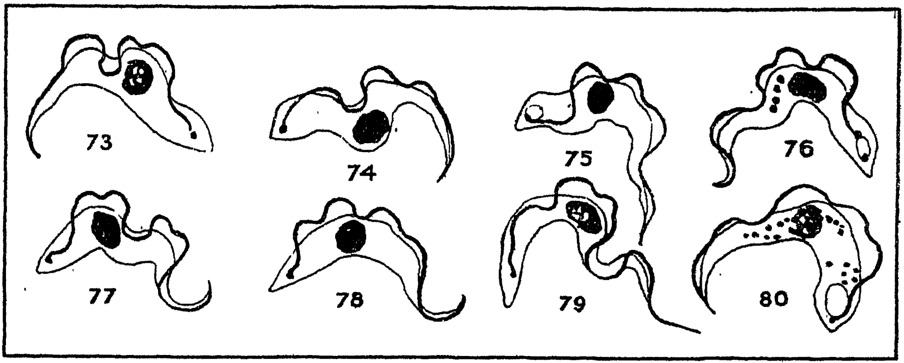
Figs. 51—59 represent various varieties of the slender type of *Trypanosoma gambiense* found in the intestine of *Glossina palpalis*. Fig. 59 is dividing. Fig. 58 is a Crithidia type. These slender and crithidial types are uncommon, and no special connection between them and the onset of infectivity in the fly has been made out.



× 2000.

Figs. 60 and 61.—*Trypanosoma gambiense* from salivary glands, 25 days after infected feed.
Fig. 62.—*T. gambiense* from salivary glands, 30 days after infected feed.
Fig. 63.—*T. gambiense* from salivary glands, 42 days after infected feed.
Figs. 64, 65 and 66.—*T. gambiense* from salivary glands, 44 days after infected feed.
Figs. 67 and 68.—*T. gambiense* from salivary glands, 46 days after infected feed.
Figs. 69 and 70.—*T. gambiense* from salivary glands, 53 days after infected feed.
Figs. 71 and 72.—*T. gambiense* from salivary glands, 56 days after infected feed.

Figs. 60—72 represent aberrant forms of *Trypanosoma gambiense* seen in the salivary glands. The first thing to be noted is the fact that the trypanosomes do not appear in the salivary glands until a late date ; in this case the earliest appearance is 25 days after the infected feed. How they reach these glands is up to the present unknown. In what shape they reach the glands is also unknown. Figs. 64, 65, 69 and 71 might be said to point to some process of segmentation, of which the finished product is represented by fig. 66.



× 2000.

FIG. 73.—*Trypanosoma gambiense* from salivary glands, 34 days after infected feed.
FIG. 74.—*T. gambiense* from salivary glands, 42 days after infected feed.
FIG. 75.—*T. gambiense* from salivary glands, 43 days after infected feed.
FIGS. 76, 77, and 78.—*T. gambiense* from salivary glands, 46 days after infected feed.
FIGS. 79 and 80.—*T. gambiense* from salivary glands, 56 days after infected feed.

When alluding, generally, in a previous part of this paper to the types of *Trypanosoma gambiense* found in the salivary glands, it was said that in the salivary glands, and here alone, the trypanosomes are found to revert to the normal type found in the blood. Figs. 73—80 illustrate this reversion. By comparing them with figs. 1, 2, and 3, which represent normal blood trypanosomes, it will be seen that they are very similar to the short and stumpy form found in the blood. No such forms have ever been seen in any other part of the fly, and we would suggest that the occurrence of these forms in the salivary glands, coinciding as it does with the renewed infectivity of the fly, is more than mere coincidence.

INFECTIVITY OF TRYPANOSOMA GAMBIENSE AFTER ITS DEVELOPMENT IN GLOSSINA PALPALIS, AS SHOWN BY THE BITES OF THE FLIES GIVING RISE TO THE DISEASE IN HEALTHY ANIMALS.

As has been shown in a previous paper,* the fly ceases to be infective by biting within a short time of its infective feed. From this time on for some 28 days the fly remains non-infective. Table II (p. 526) illustrates this.

From that Table it will be seen that in this series of experiments the flies first became infective 28 days after the infective feed, and that after this time the flies are usually found to be capable of giving rise to the disease by their bites.

It was stated above that the most important discovery made in this research is the connection between the invasion of the salivary glands and the infectivity of the fly. That this appears to be so is shown in Table III.

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* 'Roy. Soc. Proc.,' B, 1911, vol. 82, p. 498.

Table II.—The Result of the Bites of Flies at Varying Periods after an Infective Feed.

No. of days after infective feed.	Result of bites.	No. of days after infective feed.	Result of bites.
1	—	28	+
2	—	30	—
3	—	31	—
4	—	34	+
5	—	35	—
6	—	36	—
8	—	37	+
9	—	40	+
11	—	40	+
14	—	42	+
15	—	43	+
17	—	44	+
18	—	46	+
20	—	51	—
23	—	53	+
25	—	56	+

Table III.—To show Correlation between the Invasion of the Salivary Glands of *Glossina palpalis* by *Trypanosoma gambiense* and Infection by the Bite of the Fly.

Experiment No.	No of days after infective feed.	Bites of fly infective or non-infective.	Salivary glands.	Remarks.
1910	1	—	—	
1910	2	—	—	
1910	3	—	—	
1910	4	—	—	
1910	5	—	—	
1910	6	—	—	
1910	7	—	—	
1894	8	—	—	
1894	9	—	—	
2216	10	—	—	
1894	11	—	—	
1871	14	—	—	
1693	15	—	—	
1945	17	—	—	
1945	18	—	—	
1945	20	—	—	
1718	25	—	+	Blood-type not present.
1602	28	+	+	Blood-type present.
1601	30	—	—	
1945	31	—	—	
1760	34	+	+	„ „
1769	36	—	—	
1712	42	+	+	„ „
2034	43	+	+	„ „
1549	44	+	+	„ „
2034	44	+	+	„ „
1706	51	—	—	
1566	53	+	+	„ „
1651	56	+	+	„ „

From the above table it is seen that the salivary glands first become invaded 25 days after the infecting feed. In this case the monkey did not become infected. This may have been due to an accident, such as this particular fly not biting the monkey, or the invasion of the salivary glands only taking place after the fly had bitten, or to the fact that the blood-type of trypanosome was not present. With this exception all the other experiments, 12 in number, confirm the hypothesis that the fly does not become infective until the salivary glands are invaded.

Another point which comes out with striking clearness is, that in all the salivary glands from flies which gave a positive result, trypanosomes similar to the short and stumpy blood-type were invariably present.

It is to be hoped that before long the result of this work will be confirmed, and so added to, that the whole story of the development of the trypanosomes within the fly, and their passage into the salivary glands, will be unfolded.

Conclusions.

1. In the course of the development of *Trypanosoma gambiense* in *Glossina palpalis* the proboscis does not become involved, as in the case of some other species.

2. A few days after an infective feed the trypanosomes disappear out of the great majority of the flies, but in a small percentage this initial disappearance is followed by a renewed development.

3. After a very short time the flies which have fed on an infected animal become incapable of conveying the infection by their bites, and this non-infectivity lasts for some 28 days, when a renewed or late infectivity takes place.

4. A fly in which this renewed or late infectivity occurs can remain infective for at least 96 days.

5. An invasion of the salivary glands occurs at the same time as this renewal of infectivity, and without this invasion of the salivary glands there can be no infectivity.

6. The type of trypanosome found in the salivary glands when the fly becomes infective is similar to the short stumpy form found in vertebrate blood, and it is believed that this reversion to the blood-type is a *sine quâ non* in the infective process.

Preliminary Note on a Method of Measuring Colour-sensations by Intermittent Light, with Description of an Unfinished Apparatus for the Purpose.

By George J. Burch, M.A., D.Sc. (Oxon.), F.R.S.

(Received February 7,—Read March 30, 1911.)

When I began in 1895 to apply the methods of artificial colour-blindness to test the colour-sensations of other people, I cast about in various directions for means of intensifying the effect while reducing the brilliancy of the light used to produce the necessary fatigue.

The results obtained, though not immediately available for my purpose, were in several cases of sufficient interest to warrant their publication.

Successive Contrast.—In my paper on the "Relation of Artificial Colour-blindness to Successive Contrast," I described* a device whereby after fatiguing the retina with any one colour a complete spectrum might be suddenly substituted for it, thus producing "a very transient colour-blindness" which may be for the moment even more nearly complete than that described in my paper† as produced by the most intense light. For practical testing, however, this momentary effect is useless, requiring a trained eye to locate the extent of it. It forms a very good experiment in a practical course of physiological optics.

Dark-adaptation.—I found that in the dark-adapted eye the light of an ordinary lamp is strong enough to produce artificial colour-blindness to light of less intensity.‡ But there are many reasons why dark-adaptation is undesirable as a necessary condition of any colour test. Among others are the time it takes and the insuperable difficulty of the curious condition of nervousness it induces in many men, particularly among those whose colour-sensations are abnormal. I have, however, found it very advantageous to apply the spectroscopic test in a subdued light.

Simultaneous Contrast.—An attempt in 1897 to utilise the phenomena of simultaneous contrast in the examination of colour-sensations proved unsuccessful, the effect being much less striking than I had anticipated.§ But a modification of the arrangement affords an excellent method of demonstrating and explaining the phenomena.

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* 'Roy. Soc. Proc.,' vol. 66, pp. 210, 211.

† 'Phil. Trans.,' B, 1899, vol. 191, pp. 1—32.

‡ 'Roy. Soc. Proc.,' vol. 66, p. 217.

§ 'Roy. Soc. Proc.,' vol. 67, p. 226.

Intermittent Stimulation.—The very remarkable results obtained by Shelford Bidwell with intermittent light seemed capable of interpretation as due to temporary colour-blindness induced by a succession of stimuli. I therefore repeated his experiments, using the pure colours of the spectrum.* It proved possible to *see* the boundaries of the colour-sensations, by using spectral and monochromatic light instead of pigment colours for the first or for the second stimulus, but there was too much uncertainty as to which stimulus would prove effective to make the method reliable for general testing.

In May of the following year, Shelford Bidwell announced his discovery of the "External and Border Phenomena" in connection with negative after-images.† This gave the clue I wanted, showing that a very complete colour-blindness could be induced in one portion of the retina by light falling upon another part, and in my paper on "Areal Induction"‡ I described an apparatus by which this could be done. Since then my aim has been to work out the details of a practical apparatus for colour-testing on these lines.

The Apparatus.

The Spectrum.—A series of experiments with Thorp's celloidin gratings showed that though a sufficiently strong illumination could be obtained with them, the unavoidable small admixture of white light confused the results in the more refrangible parts of the spectrum. But for this objection the use of these gratings would have greatly simplified the construction of the apparatus.

With prisms, the red is so much compressed and the violet so much drawn out, that it is difficult to get a monochromatic field in the red without employing a dispersion so wide that the violet is too weak to produce fatigue. I have, however, found a way out of the difficulty.

Any pair of similar prisms symmetrically situated with regard to a beam of parallel rays may be treated as a glass prism whose angle is that between the outer faces, containing an air-prism of the angle between the adjacent faces. The pair of them together fulfil, therefore, the conditions of minimum deviation.

If, then, they are simultaneously rotated in opposite directions upon the prism table, the combination continues in the position of minimum deviation, but behaves as though it were a single prism of variable angle. Accordingly, the spectrum traverses the field of view as in a constant deviation instrument, with this added advantage, that by suitably selecting the refracting angles and the initial position of the pair of prisms, the distribution of wave-lengths in the spectrum can be varied between wide limits, so that an increased dispersion can be obtained in the red without unduly altering that of the violet.

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* 'Roy. Soc. Proc.,' vol. 66, p. 213.

† 'Roy. Soc. Proc.,' vol. 68, pp. 276, 277, etc.

‡ 'Roy. Soc. Proc.,' vol. 69, pp. 127, 128.

I used two dense-flint prisms of 60° which happened to be available, but the best distribution of wave-lengths was given by a pair of prisms of 45°, which were unfortunately too small for my purpose.

Each prism was mounted on a revolving table just big enough to hold it, and carrying a projecting arm about 20 cm. long. These arms were connected by linkwork in such a manner that any movement of one was associated with an equal and opposite movement of the other. It should be noted that for ordinary purposes no great accuracy of adjustment is needed for this part of the apparatus.

In order to get as much light as possible, and to cover a wide field of view with the slit, I adopted my usual plan of a short-focus collimator—about 25 cm.—and a long focus telescope—about 120 cm. For the second spectrum I used an instrument of the ordinary constant deviation type, of sufficient dispersion to show the sodium lines easily separated.

Light.—For all but the extreme violet Nernst lamps proved sufficient, and gave a steadier light than that of the electric arc. Save for economy in lamps, no advantage was gained by illuminating both spectroscopes with light from the same source.

Alternation of Flashes.—The stimulus was rendered intermittent by rotating discs with suitably arranged sectors. There are several positions in the path of the rays where such discs may be placed, and these were all tried. The final choice lies between two arrangements, each of which has certain advantages. In one, two discs, one to each spectroscope, are geared together by a simple arrangement of shafting that admits of the separate adjustment of each both as to position, so that they can be focussed, and as to the sequence and duration of the flashes given by them. In the other, the disc carries a mirror by which the light of one spectroscope is reflected, that of the other being seen periodically through an aperture.

Superposition of Images.—As in photometry, much is gained by making the border between the spectrum under examination and the monochromatic flash-light as nearly as possible invisible. For superposing the spectrum on the monochromatic field I have used reflection from the unsilvered surface of a prism, and have also employed an ordinary small total reflecting prism. I was investigating the relative merits of these two methods, when my illness put an end to the work.

Summary.

Briefly, the new method of measuring the colour-sensations is as follows:—

A series of flashes of monochromatic light produce by areal induction a condition of colour-blindness in a small area of the field of view, which they surround but do not invade.

This small area is occupied, also in flashes properly timed and proportioned, by the part of the spectrum under examination. The colour corresponding to that of the monochromatic flashes is, as it were, wiped out of it, and the observer sees the boundaries of the underlying colour-sensations as long as the flashes succeed one another at the proper rate.

A number of small details remain to be settled, but from what I saw when I had the model in action in 1908, I am inclined to believe that it will supersede all other methods. This apparatus was greatly superior to the first form with Thorp's gratings, figured in my paper on "Areal Induction."*

The greater part of the work was done at University College, Reading, in the intervals of teaching, but I was obliged to suspend operations altogether in 1905 in consequence of the approaching transference of the College to its new site in the London Road, and it was not until 1908 that I was able to resume the experiments. I then set up a working model of the optical parts and got out measurements for the complete instrument. These I was beginning to deal with when my health gave way, and I was obliged to limit myself to the work of the department for some time before it became necessary for me to give up altogether.

The materials of the model are now in Oxford, and I hope during the summer to superintend the putting of them in working order again.

Note.—The arrangement of shafting referred to under the head of Alternation of Flashes is as follows:—Two steel shafts, one about 4 feet long and the other 2 feet long, cross one another at right angles. They are connected by bevel gearing. On each is a sliding collar carrying a disc about 3 inches in diameter, against which a pair of discs, 9 inches in diameter, can be firmly clamped with a nut. The sliding collars can also be securely clamped to their respective shafts. In each of the larger discs two opposite sectors of 45° are cut away. By rotating one disc in front of the other effective apertures of any smaller angle can be obtained, and the apparatus being symmetrical remains balanced in all positions. These discs revolve in front of the slits of the spectroscopes, which are approximately directed at right angles to each other, and are about 3 feet 6 inches apart.

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* 'Roy. Soc. Proc.,' vol. 69, p. 127.

Spontaneous Cancer in Mice.

By M. HAALAND, M.D., Imperial Cancer Research Fund.

(Communicated by Prof. J. Rose Bradford, Sec. R.S. Received February 17,—Read March 16, 1911.)

The present paper is based upon observations and experiments made on 300 mice, obtained in the laboratories of the Imperial Cancer Research Fund since the summer of 1908, and in which cancer had developed spontaneously. Excluding lung adenomata, lymphomata, and sebaceous adenomata there remain 288 mice in which 350 tumours were observed, microscopical examination showing that they were to be regarded as true new growths. When these tumours were first observed, *i.e.* at the time of entering them on the laboratory register, 49 of the mice presented multiple tumours, and in an additional 77 mice, other subcutaneous tumour-nodules developed at a later date. Of the 350 tumours first observed, 42 had a structure quite distinctive from that of the well-known mammary tumours of the mouse, and of them 25 had certainly arisen outside the mamma; 22 were squamous-celled carcinomata with marked keratinisation, and of them 14 arose in the mammary region, and 8 entirely outside it (jaw, face, vulva, and anus); 5 were sebaceous carcinomata, and of them 3 arose in the mammary region, and 2 outside it (anus).

The remaining tumours were:—

2 Carcinomata of the preputial gland.
2 Adeno-carcinomata of the kidney (1 a hypernephroma?).
2 ,, ,, ovary.
6 Sarcomata (4 spindle cell, 1 round cell, 1 polymorphous cell).
1 Spontaneous "mixed" tumour.
1 Melanoma.
1 Fibro-myoma uteri.

(Not included in the above figures are 30 lung adenomata observed in 19 mice and cases of lymphomatous tumours.)

All the other tumours were mammary adeno-carcinomata.

The observation of typical malignant new growths in different organs provides new and additional evidence of a general liability of the tissues of the mouse to cancer, *i.e.* of an incidence in anatomical sites as varied as in man. Their malignant nature was evidenced by their clinical course, their recurrence after operation, the formation of metatases, and the results of transplantation. In short, cancer in the mouse is essentially the same process as in man.

There are, however, certain characteristic features of its incidence in the mouse attracting attention and demanding an explanation. The enormous preponderance of tumours of special organs, in particular the frequency of growths of the mamma, constitutes one of them. The frequency of multicentric development stands out as a further characteristic feature of tumours of the mamma in this animal. The data accumulated point towards an increased liability of the whole mammary system of the mouse, either as an inherited condition, or as the result of a general diseased condition, with diffuse changes in the mamma, leading to development of cancer. The same consideration applies to the adenomatous nodules in the lungs; they are also remarkably frequent and most often multiple, and to a less extent the same considerations apply to the lymphomatous tumours occurring in the mouse.

It has been generally assumed that the conditions of life of the mouse—in particular, factors connected with the physiological demands made upon the mamma, through the constantly repeated pregnancy and lactation—would suffice to account for the frequency of the mammary tumours. This point has been investigated experimentally, and the previous history of a number of mice spontaneously attacked by cancer, which have been observed since birth in the laboratory, yields no evidence of these physiological factors playing any part.

The records of the breeding experiments of the laboratory with regard to the previous history of the mice which have developed spontaneous cancer of the mamma give the following data:—Of 74 mice which had developed mammary tumours, and which were under observation from birth, only 33 are recorded to have littered previously to the development of the tumour, while no litter is recorded for 41. Analysing this latter figure, there is conclusive evidence that 20 mice have been completely isolated since quite young, and have never been with a male; and whilst the other 21 have not been so strictly isolated, but may at some time or other have been in a cage along with a male, none of them have littered. Of the 33 mice which had littered previously to the development of the tumour:—

1 litter is recorded in 11 cases.
2 litters are recorded in 7 "
3 " " 7 "
4 " " 5 "
5 " " 2 "
7 " " 1 case.

These figures show that it is hardly likely that excessive physiological demands made upon the mamma determine the development of tumours in this organ, but that other factors must be looked for.

The mammæ of a large number of old mice, cancerous and non-cancerous, have been examined histologically in serial sections, in order to investigate whether changes might be found which could account for the frequency and the multicentric origin of the tumours.

Pathological changes are very frequently met with, both in the interstitial tissue and in the epithelium. The former occur either diffusely or strictly localised, and are more or less of a chronic inflammatory character; the epithelial changes are either hypertrophic or cystic in character. The interstitial and epithelial changes are often combined. A generalised or circumscribed hypertrophic condition of the mammary epithelium occurs frequently. A general hypertrophic condition mostly indicates physiological activity following on pregnancy, and has no interest for the present purpose. Now and then such hypertrophic changes are found in a mouse which is beyond the age when a physiological activity of the mamma is to be expected, and when the mouse has been kept isolated for five or six months without a male.

The hypertrophic condition, when circumscribed, has a special interest because of the similarity of the lesions to early tumour formations. All degrees of the process are found, from a slight increase in the number of the normal acini forming one lobe to definite hypertrophic nodules. On the other hand, all gradations exist between such hypertrophic nodules and true tumours, and it is often impossible to decide between the alternatives of a hypertrophic nodule and a commencing tumour. These changes in the gland epithelium are generally accompanied by a more cellular connective tissue. They are very often multiple, and then occur either in the same mamma or in several mammæ from the same individual.

The frequency of interstitial changes—inflammation and sclerosis—in the mammæ of old mice of the laboratory is striking. There is evidence that the presence of nematodes in the subcutaneous tissue may be made responsible—at least to a large extent—for them. In several cases the interstitial changes co-exist with typical nodular hypertrophy of the epithelium, and this association suggests that the different pathological changes, inflammatory processes, sclerosis and cellularity of the connective tissue, and the nodular hypertrophy of the epithelium may be linked together, and referred to a common cause, the presence of nematodes in the subcutaneous tissue.

Thus, pathological changes of an inflammatory character are very frequent in the mammary gland of old mice. These changes have been entirely overlooked, up to the present, in the mammary gland of this animal. Along with the interstitial changes followed by sclerosis, hypertrophic changes of the glandular epithelium are frequent. In some cases the evidence favours the view that the epithelial changes are intimately connected with interstitial lesions, in others the connection cannot be definitely established. In a large number of instances the hypertrophic condition of the epithelium appears to be the basis on which tumours develop. The suggestion is made that the interstitial lesions may play an essential part as a mediate cause of the epithelial changes. An explanation of the interstitial changes described may be afforded by nematodes which have been found living in the interstitial tissue where their embryos are liberated. They are capable of causing considerable inflammatory reaction, and many of the histological pictures of inflammation and sclerosis, so often found in the mamma of old mice, can with great probability be referred to their presence. The hypothesis is put forward that the frequent and multicentric inflammation throughout the subcutaneous interstitial tissue in which the mamma is embedded may explain the frequency and the multicentricity of the epithelial hypertrophic changes and of the tumours arising on them. Similar considerations apply to the tumours of the lung and perhaps also to the lymphomatous conditions.

Numerous experiments have been made in order to define the relative parts played by local causes, such as those described above, or by constitutional anomalies of the animal in the development of cancer, or, in other words, to obtain more exact information on the relations obtaining between the tumour-cells and the animal in which they arose. The observations thus made may be considered under the following heads:—

(*a*) Inoculations into the same mouse in which the tumour originated (autologous or autoplastic implantation).

(*b*) Inoculations into other spontaneously attacked mice.

(*c*) Inoculations into normal mice, young and old.

(A) *Autologous Implantation.*

The tumours employed and the results obtained are:—

		Positive.	Negative.
51 adeno-carcinomata of mamma—			
	46 subcutaneous	44	2
	5 intraperitoneal	5	
2 adeno-cancroids	subcutaneous	2	
5 squamous cell carcinomata	„	5	
1 sebaceous carcinoma	„	1	
3 spindle-celled sarcomata...	„	3	
1 melanoma	„	1 ?	

Out of 59 epithelial tumours of varied histology, 57 have grown on transplantation of the cells into the same spontaneously affected animal.* Of non-epithelial tumours there were four, three spindle-celled sarcomata which gave positive results on re-inoculation, for the fourth (a melanotic tumour of peculiar structure and of very low power of growth) no increase was noted of the grafts re-inoculated simultaneously in four different places into the mouse in which it had arisen, although the tumour cells remained alive for four months.

(B) *Inoculations into other Spontaneously Affected Animals.*

Of the tumours which have been re-implanted into the same mice in which they have arisen, a number have also been tested in other mice spontaneously attacked with cancer. The method of implantation, dose, and site were exactly the same as for the autologous implantations.

Fifty-eight implantations have been made, and of them five have been successful. The tumours employed and the details of the results obtained, as compared with the results in normal mice, will be seen from table appended.

(C) *Inoculations into Normal Mice, Young and Old.*

In all, 73 tumours have been transplanted into 7706 mice, of which 5519 have survived four weeks; 307 tumours have been obtained, *i.e.* an average of about 5·6 per cent.

The experiments recorded above show that, whereas the spontaneously attacked animal offers a much more favourable soil for the growth of its own tumour cells, it is very doubtful whether it offers any better conditions for

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* The success attending autoplastic implantation is not limited to tumours of the mouse. It affords a method for obtaining fresh material wherever difficulties arise in transferring the tumours to normal animals, especially valuable in the case of larger animals or with ulcerated tumours, and is thus practised in the laboratory.

Nature of tumour transplanted, and protocol number of the mouse bearing it.		Into mouse itself.	Into other spontaneously affected mice.	Into Normals.	
				Young.	Old.
Cancroid	266	+	0/2	0/349	
„	273	+	0/1	0/337	
„	349	+	1/7	5/180	
„	357	+	0/4	1/59	
Sebaceous carcinoma	292	+	0/3	4/42	
Adeno-carcinoma. Mamma	282	+	2/4*	4/26	0/12
„ „	322	+	2/7	8/52	
„ „	334	+	0/5	0/16	0/16
„ „	338	+	0/5	2/60	0/15
„ „	351	+	0/1	0/35	
„ „	353	+	0/1	3/35	
5 other adeno-carcinomata	5	+	0/10	Not tested	in normals
Spindle cell sarcoma	460	Not transplanted; mouse killed	0/8	16/130	0/15
Total		16/16	5/58	43/1321	0/58

* 2/20 when all inoculations are reckoned.

growth of other spontaneous tumours than do normal animals. The question of the suitability of the soil afforded by cancer-mice to the growth of tumours is a most important one. In order to study this question a little more closely, the behaviour of cancer-mice to different transplantable tumours has been investigated. Five experiments with 46 spontaneously affected mice have been undertaken with four different tumours representing different types of growth.

In order to employ control animals as comparable as possible to the cancer-mice, cancerous mice from the breeding experiments of the laboratory have been used, and as controls, their sisters and other near relatives kept under exactly the same conditions and of the same age as the cancerous mice.

The results of a typical experiment are seen from the chart (fig. 1), done with a rapidly growing carcinoma which usually grows progressively in a high percentage (Carcinoma 63). Expressed in figures, the result is, in young normals, six tumours in ten mice, *i.e.* 6/10; in old normals, 5/10; in old non-cancerous females of the breeding experiments of exactly the same ages as the cancerous mice, 2/10 (*plus* three positive cases which died too early to settle definitely whether the tumour would have grown progressively); and in cancer-mice from the same breeding experiments, 4/7.

On the whole, these experiments have failed to elicit any striking difference in suitability between spontaneously affected and normal animals. As in all transplantations of tumours, young animals show a greater susceptibility than older ones. In most of the experiments the transplanted

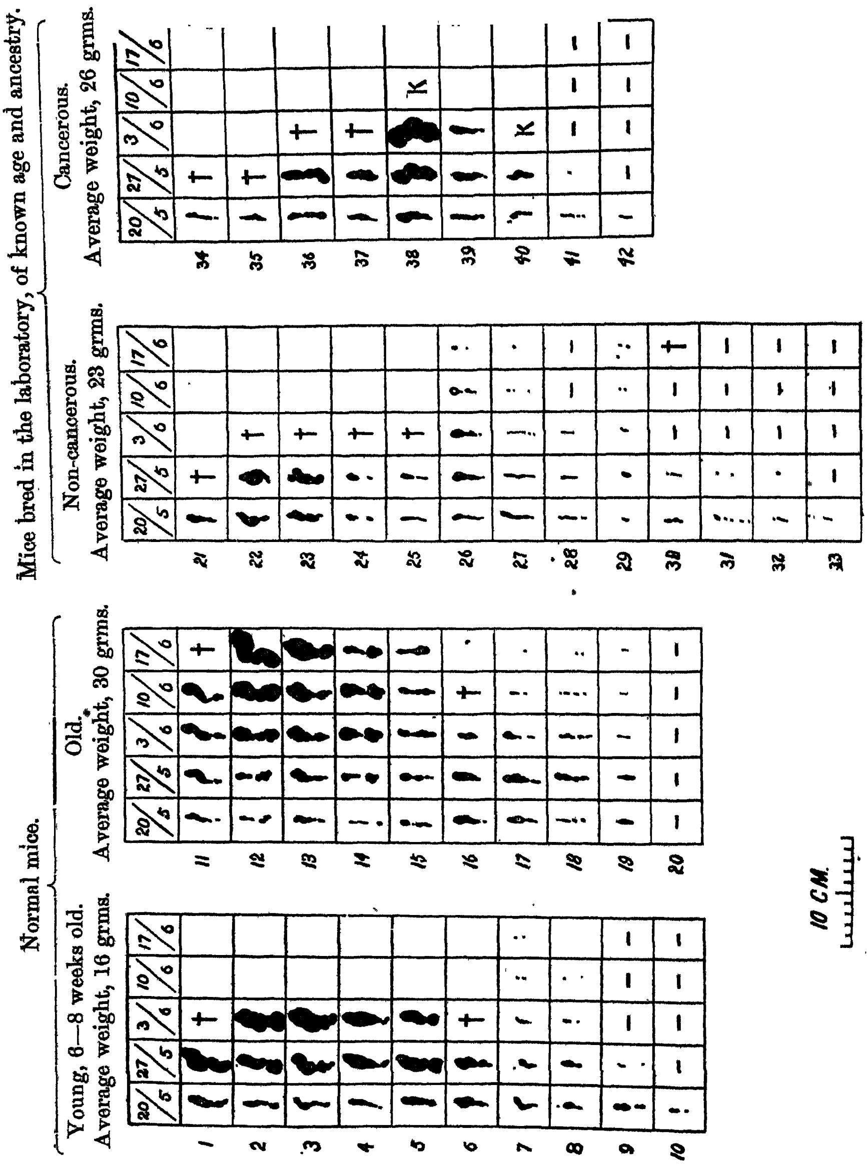

FIG. 1.—Suitability for the Growth of Transplanted Cancer of Mice in which Cancer has developed spontaneously and has been removed by operation as compared with non-cancerous mice.

All mice inoculated 10.5.10 in the right axilla with 0·02 c.c. of Carcinoma 63. (Exp. 63/45 B.) Mice 1—33 controls (see headings of columns); 36—42 cancerous mice.

tumours have grown better in the young control mice than in the spontaneously affected animals. When the cancer-mice are compared with old mice of the same age, there seems to be a difference in some experiments in the case of certain tumours in favour of the former. This is the case in the experiment recorded on the chart (fig. 1). A similar difference seems to be present in the results of implantation of some spontaneous tumours (*vide* Table, p. 537). In other experiments, hardly any such difference can be discovered, while in some the growth in the spontaneously affected mice was behind that in the old normal mice. The differences which exist in the two first-mentioned experiments are small, and, keeping in view the fact that the conditions in spontaneously affected and in normal mice, even at the best, are not strictly comparable, there does not seem to be evidence of an essentially enhanced suitability for growth of cancer cells in general, in mice spontaneously affected with cancer as compared with normal mice.

The experiments show that growth nearly always follows when a fragment of a spontaneous tumour is re-introduced into the animal in which the tumour has arisen, whereas the result is positive only in a very small percentage of normal animals. A general or constitutional idiosyncrasy favourable to the growth of its own cancer seems to be present in the spontaneously attacked animal, but it only applies to cells of its own body and not to cancer cells in general. On investigating spontaneously affected cancer-mice from the standpoint of the soil they offer for the growth of tumour cells which have originated in other individuals, one finds that the conditions then do not differ much from those obtaining in normal animals. The same inference can be drawn from the results obtained when cancer-mice are tested as to their suitability for the growth of propagated tumours, and the result compared with that obtained in normal mice under conditions as similar as possible. The comparison demonstrates that there is no evidence of general constitutional changes in the spontaneously attacked animals, greatly favourable to the growth of cancer cells in general, which can explain the appearance of a cancer. The results of autologous implantations are to be explained by the fact that the cells introduced belong to the same organism, where they have the full command of the supply of vessels and supporting tissues, and because they can be reintroduced either without setting up any inimical reaction, or if a reaction directed against them is set up, being in congenial surroundings, they are indifferent to it.

Experiments have been made to ascertain if reactions similar to those observed in normal mice against propagated tumours take place in spontaneously attacked mice, and if they do, whether they are efficient against their own tumour. Cancer-mice are capable of bringing transplanted tumours to absorption in the same way as normal mice do, and there is no reason to assume that the reactions leading to absorption differ essentially in spontaneously attacked mice and in normal mice, when the tumour cells introduced are derived from another individual. In some rare instances there is evidence that a process of spontaneous absorption or healing may also take place in a mouse towards its own spontaneous tumours, but it is very rare, and the evidence points rather to the importance of local than to general or constitutional changes. When an attempt is made to induce increased resistance in spontaneously attacked mice, by the same means as are successful in normal mice against implantation of cancer, they seem to be totally ineffective against the implantation of the spontaneous tumour in the mouse in which it has arisen. There seems to be an insensibility of the cell in its own animal to reactions which are effective against strange cells in the same surroundings.

The outstanding result of this inquiry is the production of evidence of possible *local causes* of origin, on the one hand, and the absence of *general constitutional changes* favourable for growth on the other. The importance of local factors for other forms of cancer has long been recognised, and has been drawn attention to repeatedly in previous publications of the Imperial Cancer Research Fund. As knowledge of them increases, their many-sided nature appears more and more clearly. Whether they be animate or inanimate, bilharzia, nematodes, growth of other cancer cells as in the development of sarcoma as in Mouse 469, or mechanical, chemical, and actinic influences, the ultimate result of their action may be the same—the creation of new cell strains with powers of continuous growth. On the one hand, this process of cancerous transformation shows a close association with chronic irritative changes; on the other it has many points in common with what is known as spontaneous variation, giving rise to sports in animals or plants. The part played by the chronic irritant is obviously a mediate one, either so that it produces the altered conditions under which the first departure of the cell from the normal may take place, or by giving spontaneously occurring sports of cells opportunity of multiplying and by degrees adapting themselves to a new mode of life, similar to what is observed in propagated tumours. This hypothesis rests on the assumption that the malignant transformation of the tissues may take place by stages, for which there now seems to be ample evidence both from the facts obtained from observation of spontaneous cancers and from those obtained in propagated tumours. The nature of this process is still unknown, but it is possible that it is being elucidated indirectly by the study of the variations which, as recorded in another paper, have been demonstrated to occur in cancer cells during their continued propagation. The hope of being able to investigate the nature of the process by more direct methods lies in the possibility of being able to reproduce experimentally the conditions under which it may take place.

On Variation and Adaptation in Bacteria, Illustrated by Observations upon Streptococci, with Special Reference to the Value of Fermentation Tests as Applied to these Organisms.

By E. W. Ainley Walker, M.A., D.M., Fellow and Tutor of University College, and Lecturer in Pathology in the University of Oxford.

(Communicated by Prof. Francis Gotch, F.R.S. Received February 18,—Read March 30, 1911.)

(From the Department of Pathology, University of Oxford.)

The fermentation tests introduced by M. H. Gordon (1) in his study of streptococci isolated from saliva and some other sources were proposed by him as a means of differentiating among these organisms and separating them into definite varieties.

Gordon's methods were applied by A. C. Houston (2) in observations upon streptococci obtained from normal human fæces and from milk, and they were largely relied on by Andrewes and Horder (3) in their investigation of pathogenetic streptococci from disease in man.

The tests in question concern the reaction (acid or alkaline) produced by any given streptococcus during three days' growth at 37° C. in a series of tubes of alkaline sugar-free litmus broth to which 1 per cent. of saccharose, lactose, raffinose, inulin, salicin, coniferin, and mannite respectively have been added. The production of acid in sufficient amount to change the colour of the medium from blue to red constitutes a positive reaction in each case, and is believed to be due to the fermentation of the added carbohydrate substance by the streptococci.

To these culture tests in the seven different carbohydrates two others were added by Gordon, namely, the presence or absence of clotting in litmus milk within three days at 37° C., and the reduction or non-reduction of neutral red broth in anaërobic culture at 37° C. within two days.

In Houston's observations the coniferin test was omitted, and so far as I am aware this glucoside can now no longer be obtained commercially. It was, however, employed by Andrewes and Horder, who, as the result of their investigations, maintained their ability to distinguish well-marked differences of type among their streptococci. These observers elaborated a method of classification based on Gordon's tests, but taking into consideration in addition the length of the streptococcal chains produced in ordinary broth (at 37° C.), and the power of growth in gelatin at 20° C. with or without the liquefaction of that medium. It would appear that they regard their method as enabling them to subdivide the morphologically indivisible type, Streptococcus, into a number of distinct and independent groups or "provisional species."

It is clear that so simple a method of distinguishing definite varieties (if such exist) among the streptococci would possess the greatest value, not only from a theoretical and taxonomic standpoint, but also from its enormous practical utility and most important bearing both on the diagnosis, the prognosis, and the treatment of streptococcal infections. But while a method of such great potential value cannot be lightly set aside or disregarded in dealing with the organisms in question, neither can it be accepted with reliance without the most thorough and complete investigation.

The problem of the identity or diversity of the streptococci which invade the human body is one whose importance is almost equalled by its difficulty. Many observers have attacked the problem without conclusive result, and Marmorek's (4) contention for the unity of human streptococci still held the field successfully at the period when Gordon's tests were introduced.

The question had been prominently before my own mind, owing to the necessity of endeavouring to determine whether the micrococcus which (following Triboulet and Paine and Poynton) I found in a number of cases of acute rheumatism, and spoke of as the *Micrococcus rheumaticus*, was or was not distinguishable from other members of the group Streptococcus. Such definite distinction it was eventually found impossible to establish, since even the remarkable production of formic acid by the streptococcus from rheumatism which Ryffel and I (5) observed proved to be only quantitative and not qualitative in character. I was therefore anxious to determine whether Gordon's tests would afford a reliable means of differentiation.

Clearly the first requisite for test reactions such as those proposed is that they shall yield constant and definite results with any given strain of streptococcus. Gordon himself fully recognised that "stability of the reactions" was important, and gave some attention to the question, but regarded it as sufficiently decided when he had ascertained that the reactions remained unchanged in observations extending over a fortnight, and were only modified in two cases out of eleven (and then only in respect of one or two of the reactions) by one passage of the micro-organisms through the mouse.

In their preliminary criticism of Gordon's tests, Andrewes and Horder also emphasised the fact that "constancy of the tests is clearly a cardinal point," and they went on to state that as the result of their own observations they had "no hesitation in asserting that the reactions are remarkably constant" for any particular streptococcus examined. They admitted that the neutral red and salicin reactions showed some tendency to variation, and mentioned two instances in which the mannite reaction was lost after a week of culture on gelatin. But on the whole they found the tests appreciably constant. They stated that they had in a number of cases found them unchanged after so long as a year, quoting one instance in which the interval of observation was nine months. Other evidence which they gave of constancy is open to the criticism that it consisted in part of examples in which the reactions actually showed some degree of variation, and they accordingly suggested that "slight differences in the composition of the media may possibly affect the series of reactions to some little extent." Evidence is offered here that greater differences in the media do actually affect the reactions to a remarkable extent.

Using these test reactions Gordon found as many as 48 different chemical types among 300 streptococci from saliva, though it is to be noted that more than one-third of the number fell under three of the types. Houston found 40 types among 300 streptococci from fæces.

Andrewes and Horder, examining 228 strains of streptococci from man, referred them all to five types (one of which consists of pneumococci), but this was only accomplished by classifying no less than 173 of the strains as variants by defect and excess of one or other of the five main types selected. Their streptococcal types are named respectively pyogenes, salivarius, anginosus, and fæcalis.

The remarkable frequency of variants and the numerous gradations of the type forms are explained by Andrewes (if I understand him rightly) as due to the survival and persistence of intermediate biological links, connecting those main types in the "dominant genus" constituted by the streptococci, which will become distinct species if (and when) these variants disappear in the course of evolution. This view entails the supposition that the types and variants in question have definite and relatively fixed identities, and that the characters relied on for their differentiation have to some extent at any rate a specific significance. But it would seem to be an equally satisfactory alternative in an *a priori* explanation to suppose that the differences observed in the chemical reactions now under consideration are due to merely temporary and casual modifications of metabolism. It might further be suggested that the features of streptococcal activity which they concern are so extremely variable, and are so easily varied under the influence of varying environment as to be almost accidental in character and to afford no criteria of any differential value, nor any basis for a classification of these micro-organisms. This is the attitude which my experiments appear to justify.

Abundant grounds exist in the known variability of chemical activity, and even of morphological characters which bacteria present, for regarding any simple test for specificity among these organisms with some suspicion, until it has been fully investigated under appropriate conditions. Under the influence of altered environment a considerable degree of adaptation would naturally be anticipated in these primitive organisms where the successive generations follow one another with such great rapidity as to reach in many cases even two or three per hour. But the remarkable degree of variability which bacteria do actually exhibit is perhaps not always kept sufficiently in mind.

The great difficulties met with by Dreyer and FitzGerald (6) in ascertaining the precise conditions under which neutral red lactose bouillon could be made to yield uniform results in the differentiation of the *B. typhosus, B. paratyphosus,* and *B. coli* showed incidentally that slight alterations in the composition of the culture medium are frequently sufficient to modify and even to *reverse* the chemical changes produced by these organisms in the medium in question.

Again, Twort (7) has shown that the *B. typhosus* can be trained (in about two years) to ferment lactose in lactose peptone water. More recently Penfold (8) has noted a morphological change in the colonies associated with the development of this faculty, and has made important observations on the development or loss under suitable conditions of a number of other fermenting properties of the same organism. My own earlier observations (9) on the "immunisation" of the same bacillus against its own specific immune serum (of the horse) by continued cultivation and sub-cultivation in that fluid demonstrated an adaptation of much greater complexity. This was associated with an increased virulence of the micro-organism, an increased resistance to the specific action of the anti-serum, and what appeared to be an actual production of specific anti-antibodies, showing a high degree of adaptability in the organism in question.

Morphological variations of a greater or less degree are also common, and like the chemical variations they can be induced experimentally. Almquist (10) has described them both in *B. typhosus* and in *Vib. choleræ*; and Murray and I (11) have shown that in special media the *B. typhosus* can be made to assume a variety of novel appearances, in some of which it might readily be mistaken for a streptothrix.

Both morphological and chemical variations of greater or less permanence are, therefore, very readily produced among bacteria. Accordingly a claim put forward that particular strains of any given type of micro-organism, such as Streptococcus, can be regarded as distinct and fixed varieties on the results of any given series of culture tests requires to be most carefully scrutinised before it can be accepted, and the constancy of the reactions concerned must be thoroughly assured.

As regards the streptococci, however, the observations which I have carried out appear to show that this is not the case. If cultures of these organisms are examined at considerable intervals of time their reactions in the test media are by no means constant, but on the contrary are found to undergo remarkable changes. Further it is found that such comparatively simple manipulations as continued culture in a particular medium may entirely alter the reactions of these organisms not only to that medium itself, but (it may be) to others also of the carbohydrate media.

METHODS.

The streptococci to be examined were first plated out twice to ensure as far as possible that the culture obtained arose from a single individual or chain of individuals. With the exception of the one named E below (cultivated from horse-dung) they had all been isolated from the human subject.

The stock cultures of these organisms were propagated in stab agar, and sub-cultures were established every seventh day.

The test media were prepared as directed by Gordon, at first with ordinary bouillon freed from sugar, but later, and for the bulk of the results recorded below, from "lemco." With each series of cultures in a particular test medium an uninoculated control tube of the same medium was put up, and incubated with them for comparison. Whenever a negative reaction was obtained, a sub-culture from the negative tube was prepared in ordinary bouillon in order to make sure that the organism had actually grown in the test medium. If growth was not obtained in this sub-culture, the test was repeated.

In the tables given below, the sign + indicates the production of an acid reaction under the test conditions (and where milk is concerned, the formation of a clot), while the sign 0 indicates that the reaction remained alkaline (or milk unclotted, whether it remained alkaline or became acid in reaction). The former may be spoken of as a positive, and the latter as a negative reaction. The neutral red test, which is admitted to be somewhat variable, was usually omitted; and coniferin, which could not be obtained commercially, was not used.

RESULTS.

In the earlier observations I examined a considerable number of streptococci of known origin which were available at different times, and noted their reactions, subsequently comparing the results thus recorded with those obtained after a longer or shorter period of cultivation in stab agar as laboratory stock. Three sets of observations illustrate the character of the results thus obtained:—

Table I.—Streptococcus B. Showing Six Changes of Reaction between June, 1907, and May, 1908.

Date of testing.	Milk clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Changes.	Total of changes.
June, 1907	0	+	0	0	0	0	+		
January, 1908	0	+	+	+	0	0	0	3	
May, 1908	+	+	0	+	+	0	0	3	6

Streptococcus B.—This was isolated from a case of acute rheumatism by Dr. Bushnell, in 1907, and sent to me for examination of its formic acid production.

Table II.—Streptococcus E. Showing Two Changes of Reaction after Three Months' Cultivation in Stab Agar.

Date of testing.	Milk clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Changes.
August 8, 1910	0	+	+	0	+	+	+	
November 15, 1910.....................	0	+	+	+	0	+	+	2

Streptococcus E, tested when freshly isolated from horse-dung, and again after 14 weeks.

Table III.—Streptococci L, P, S, G, M, H and V. Showing the Changes of Reactions (20 out of a possible 49) which had taken place after the lapse of Two Years.

Streptococcus.	Date of testing.	Milk clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Reactions gained.	Reactions lost.	Total of changes.
L	June, 1908 ...	+	+	0	0	0	0	0			
	„ 1910 ...	0	0	+	0	0	+	0	2	2	4
P	„ 1908 ...	+	0	+	+	0	+	0			
	„ 1910 ...	0	0	+	0	0	+	0	0	2	2
S	„ 1908 ...	+	+	+	+	0	+	+			
	„ 1910 ...	0	+	+	0	0	+	0	0	3	3
G	„ 1908 ...	+	+	+	0	+	+	+			
	„ 1910 ...	+	+	+	+	0	+	0	1	2	3
M	„ 1908 ...	+	+	+	+	0	+	0			
	„ 1910 ...	0	+	+	0	0	+	0	0	2	2
H	„ 1908 ...	0	+	+	+	+	+	0			
	„ 1910 ...	0	+	+	0	0	+	0	0	2	2
V	„ 1908 ...	+	+	0	0	+	+	0			
	„ 1910 ...	0	0	+	0	0	+	0	1	3	4

Seven streptococci selected as giving each a different series of reactions when first tested in 1908. L and P had been under cultivation in the laboratory for more than a year, while S, G, M, H, and V had recently been introduced from Copenhagen.

These results show that, when examined after longer or shorter intervals of time, the reactions of the streptococci tested exhibited marked changes under the conditions of ordinary cultivation.

The seven strains of streptococci shown in Table III were now chosen for further investigation.

P, which had never fermented saccharose, was grown in the saccharose medium.

L and V, which had never fermented raffinose, were grown in the raffinose medium.

M, V, and H, which had never fermented mannite, were grown in the mannite medium.

The cultures were sub-cultivated every three days in the appropriate medium. The course of the experiments was as follows:—

P in Saccharose.

June 14—17.........	1st passage	Reaction 0
„ 17—20.........	2nd „	„ +
„ 20—23.........	3rd „	„ 0
„ 23—26.........	4th „	„ +
„ 26—29.........	5th „	„ +

The negative reaction reappeared occasionally until the middle of July, after which the organism remained a strong saccharose fermenter until the end of the observation (August 13). It was then tested through all the media, but showed no change in any of its other test reactions.

L and V in Raffinose.

Begun June 14.

V became a raffinose fermenter on the fifth passage and so remained as long as it was kept under observation (several weeks).

L refused to ferment raffinose, though grown continuously in this medium until October 7. It was then carried on *anaërobically* in the raffinose medium, and began to change the colour to red on the third passage. This reaction continued to be obtained in successive passages, though until October 27 the *blue colour was found to return on admitting air.* From October 27, the red colour produced anaërobically remained permanent even when air was admitted. But on continuing the cultures aërobically from November 10 to December 5, the organism still refused to ferment the raffinose in the presence of oxygen.

The change of colour to red produced anaërobically, which speedily changed back again to blue on admitting air, has frequently been met with in other instances, and appears to be quite common. Still more frequently the blue colour is simply discharged in the anaërobic cultures. In a large number of cases the blue colour rapidly returns on the admission of air, reappearing first at the surface of the medium and travelling slowly downwards to the foot of the tube. The medium will frequently be found to be still quite strongly alkaline in reaction.

The change concerned in this discharge of colour must apparently be one involving some deoxidation process, as the result of which the litmus is converted into a leuco-body, but I am at present quite unable to throw any light upon the actual nature of the change. It is, however, important that it should not be mistaken for an anaërobic fermentation of the test carbohydrate.

As Andrewes pointed out, such fermentation may often be obtained quite readily in anaërobic cultures, when it entirely fails to appear under aërobic conditions of cultivation.

M, V, and H in Mannite.

H became a mannite fermenter on the third passage, and so remained. M and V were carried on in mannite from June 14 until August 1 without any change in the reaction appearing.

These three sets of observations show that by continued passage through a particular medium some of the streptococci easily acquire the faculty of fermenting a special carbohydrate, to which they had always previously given a negative reaction. In other cases similar changes could not be obtained within the period of investigation.

Inulin Test.

In the next set of experiments inulin was selected as the medium for observation, as being apparently the most difficult for streptococci to ferment. Andrewes and Horder found that only 3 per cent. of their streptococci could ferment this polysaccharide.

Five streptococci, L, P, S, G, M, were selected for observation, none of which fermented inulin, and only one of which (G) had ever done so within my experience. Table IV shows the result of growing these organisms for certain periods in inulin medium. All became eventually (eight weeks) strong inulin fermenters. They were then put back into stab agar for three months, after which all but M were found to have lost their action upon

Table IV.—Streptococci L, P, S, G and M. Showing the Reactions in Inulin Medium at Different Stages of the Experiment.

Streptococcus.	Original reaction in inulin.	After 14 days in inulin medium.	After 24 days in inulin medium.	After 5 weeks in inulin medium.	After 8 weeks in inulin medium.	After return for 3 months to stab agar.	After 4 weeks' anaërobic culture and 2 weeks' aërobic culture in inulin medium.
L	0	0	+	+	+	0	+
P	0	0	0	+	+	0	+
S	0	0	+	+	+	0	+
G	0	0	0	0	+	0	0
M	0	+				+	+

inulin. On again growing them, first anaërobically (four weeks) and then aërobically (two weeks) in inulin medium, all regained the power of acting anaërobically on inulin, and all but G the power of acting on it in the presence of air.

At intervals during the course of this experiment the organisms from inulin culture were sub-cultivated, and tested through the series of test media, with the results given in Table V below. They showed marked variations at different stages of the experiment.

Table V.—Streptococci L, P, S, G and M. Showing the Reactions of the Five Streptococci in the Test Media at Different Stages of the Inulin Experiment.

Streptococcus.	Time of testing.	Milk clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Changes.	Total of changes.
L	1. Original reactions, June, 1910	0	0	+	0	0	+	0		
	2. After 5 weeks in inulin	0	+	+	0	+	+	0	2	
	3. After 8 weeks in inulin	+	+	+	+	+	+	+	3	
	4. After 3 months in stab agar	0	0	+	0	0	+	0	5	
	5. After 2 weeks in inulin (anaërobic)	+	0	+	0	0	+	+	2	
	6. At end of experiment	0	0	+	0	+	+	0	3	15
P	1. Original reactions, June, 1910	0	0	+	0	0	+	0		
	2. After 5 weeks in inulin	0	0	+	0	+	+	0	1	
	3. After 8 weeks in inulin	0*	0	+	0	+	+	0	0	
	4. After 3 months in stab agar	0	0	+	0	0	+	0	1	
	5. After 2 weeks in inulin (anaërobic)	+	0	+	0	0	+	+	2	
	6. At end of experiment	+	0	+	0	+	+	0	2	6
S	1. Original reactions, June, 1910	0	+	+	0	0	+	0		
	2. After 5 weeks in inulin	0	+	+	+	+	+	0	2	
	3. After 8 weeks in inulin	+	+	+	+	+	+	+	2	
	4. After 3 months in stab agar	0	0	+	0	0	+	0	5	
	5. After 2 weeks in inulin (anaërobic)	+	0	+	0	0	+	0	1	
	6. At end of experiment	+	+	+	+	+	+	0	3	13
G	1. Original reactions, June, 1910	+	+	+	+	0	+	0		
	2. After 5 weeks in inulin	0	+	+	+	0	+	0	1	
	3. After 8 weeks in inulin	+	+	+	+	+	+	+	3	
	4. After 3 months in stab agar	0	+	+	0	0	+	0	4	
	5. After 2 weeks in inulin (anaërobic)	0	+	+	0	0	+	+	1	
	6. At end of experiment	+	+	+	+	0	+	+	2	11
M	1. Original reactions, June, 1910	0	+	+	0	0	+	0		
	2. After 2 weeks in inulin	0	+	+	+	+	+	+	3	
	3. After 3 months in stab agar	0	+	+	+	+	+	+	0	
	4. After 2 weeks in inulin (anaërobic)	0	+	+	+	+	+	+	0	
	5. At end of experiment	0	+	+	+	+	+	+	0	3

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* After another week of cultivation in inulin medium this reaction became positive.

It will be noted that, after eight weeks of growth in inulin, L, S, and G gave identical reactions which only differed in respect of the milk-clotting from the reactions given by M after a fortnight's growth in the same medium. The number and variety of the other changes which appeared is very striking.

Mannite Test.

An attempt was also made to teach the organisms, L, P, S, G, M, V, H, to ferment mannite by growing them in the mannite medium. This was unsuccessful after four months' trial, but is interesting as showing (Table VI) that although the mannite reaction itself refused to change, some of the other reactions were modified in some cases by the continued cultivation in mannite.

The following is the protocol of the experiment:—

L, P, S, G, M, V, H, were grown from August 1 to October 1 as follows: Three days in mannite medium, sub-culture in ordinary bouillon one day, back to mannite medium for three days, and so on. No change of reaction had occurred by October 1.

The organisms were now grown three days in mannite anaërobically, one day in bouillon aërobically, back to anaërobic culture in mannite for three days, and so on. By October 15 all but G and H discharged the colour in mannite, but the blue colour returned on admitting air. This state of things continued in the successive cultures until November 10, when G and H were abandoned. L, S, P, V, M, were now grown in mannite

Table VI.—Streptococci L, P, S, V and M. Showing the Changes of Reactions which had taken place at the end of the Mannite Experiment.

Streptococcus.	Time of testing.	Milk clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Changes.
L	1. June, 1910	0	0	+	0	0	+	0	
	2. After 4 months in mannite	0	0	+	0	0	+	0	0
P	1. June, 1910	0	0	+	0	0	+	0	
	2. After 4 months in mannite	0	0	+	0	0	+	0	0
S	1. June, 1910	0	+	+	0	0	+	0	
	2. After 4 months in mannite	0	+	0	0	0	0	0	2
V	1. June, 1910	0	0	+	0	0	+	0	
	2. After 4 months in mannite	+	0	+	0	0	+	0	1
M	1. June, 1910	0	+	+	0	0	+	0	
	2. After 4 months in mannite	0	0	+	0	0	+	0	1

aërobically, sub-cultures being made every three days directly from the mannite cultures to fresh mannite media.

On December 5 all were still negative to mannite. The organisms were now tested through the other test media, with the results shown above in Table VI.

Milk Test.

The streptococci L, P, S, G, M, were grown continuously in milk for well over four months, from June 17 until October 31, fresh subcultures being made in litmus milk once a week.

When the experiment was begun only G clotted milk (on the second day).

On July 15 S and P had begun to clot milk, while G now only clotted the milk on the fourth day of growth.

On July 21 G had ceased to clot milk at all, and had not regained this power by October 31, when the experiment was brought to an end.

On August 19 S and P no longer clotted milk. On September 20 S gave a feeble clot. On October 2 L and P clotted the milk on the sixth day. On October 19 L, P, and S clotted the milk firmly in three days, and this condition still held on October 31. M never clotted milk throughout the experiment.

Thus, of the five streptococci, one lost the power of clotting milk, three gained it, and one remained unchanged. These five organisms were now put

Table VII.—Streptococci L, P, S, G and M. Showing the Changes of Reactions which had taken place after Four Months' Cultivation in Litmus Milk.

Streptococcus.	Time of testing.	Milk clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Changes.
L	1. June, 1910	0	0	+	0	0	+	0	
	2. After 4 months in milk	+	0	+	+	+	+	0	3
P	1. June, 1910	0	0	+	0	0	+	0	
	2. After 4 months in milk	+	0	+	0	+	+	0	2
S	1. June, 1910	0	+	+	0	0	+	0	
	2. After 4 months in milk	+	0	+	+	0	0	0	4
G	1. June, 1910	+	+	+	+	0	+	0	
	2. After 4 months in milk	0	+	+	+	0	+	0	1
M	1. June, 1910	0	+	+	0	0	+	0	
	2. After 4 months in milk	0	+	+	0	0	+	+	1

through the test media, and showed other changes of reaction as exhibited in Table VII above.

The general bearing of the results obtained in the experiments recorded above on the problem of the identity or diversity of human streptococci, and on the value of test media in the diagnosis and differentiation of these organisms may now briefly be indicated.

The streptococci shown in Tables I, II, and III undergo such great changes under cultivation in stab agar as to exhibit collectively 28 alterations of individual reactions out of a possible 70, namely, nine gains out of a possible 28, and 19 losses out of a possible total of 42.

Among the seven streptococci in Table III which each gave a different series of reactions in 1908, and could therefore at the date have been classified as different varieties, L, P, and V give identical reactions in 1910, and S, M, and H are also identical, but give a slightly different series of reactions from the former, while G still stands alone, although itself somewhat changed. Examined for the first time at this date these seven streptococci might, therefore, have been regarded as representing only three varieties.

At the end of the mannite experiment (Table VI) M is no longer identical with S, but has assumed the reactions of the L, P, V group, while V has left this group and now clots milk.

Streptococcus G, after three months in agar following eight weeks in inulin (Table V), gives the same reactions as M (vi, 1910) in Table III. And L, S, and G, in Table V, give identical reactions at the third stage—all positive, and only differ from M in that the latter still lacks the power of clotting milk, which it possessed when first observed in 1908.

It is unnecessary to adduce further instances, though a number occur in the tables, that streptococci, which are at one time different, may at another give identical reactions, while those which are apparently identical at a given date may later on exhibit totally different series of reactions in the test media. But it is clear that no trace of specificity has been found in the tests in question in the present observations.

Looking through the tables one finds that Streptococcus L exhibits no less than seven different series of reactions in different circumstances, P exhibits six different series, S exhibits nine, G exhibits seven, and M exhibits four. These five streptococci have, in fact, given rise to 32 different varieties or variants in the course of this investigation. It follows that the method of identifying varieties of streptococci by means of the series of test media which have been employed in these experiments rests on no fixed or specific differences in the organisms themselves. The differences observed cannot in any sense be regarded as permanent, but would seem to be due to merely temporary changes in the metabolism of the organisms concerned.

Lest it should be thought that the alterations in reactions which have been here described are not of absolutely fundamental character, I have compared a number of my induced varieties as nearly as possible with the types and variants described by Andrewes and Horder. For this purpose it is necessary to omit their neutral red and coniferin tests, which I have not used, and to disregard the questions of growth in gelatin and length of chains in bouillon. These latter do not appear to form very important features in their system of classification. The facts, however, must be fully borne in mind in estimating the value of the present criticism. Their streptococcal types are numbered (1) pyogenes, (2) salivarius, (3) anginosus, and (4) fæcalis; the variants being indicated by the letters *a*, *b*, *c*, *d*, etc.

Among my streptococci derived from L are to be found (with the provision just mentioned) the following types and variants of Andrewes and Horder in succession:—3 *c*, 1 *d*, 3 *g*, 4 *b*, 1 *d*, 4 *e*, 3 *z*, 1 *d*, and a form not met with by these writers. That is to say, L appears to correspond at different times in my experiments with variants of *S. pyogenes*, *S. anginosus*, and *S. fæcalis* in their classification. P gives 1 *a*, 3 *h*, 1 *d*, 3 *e*, of Andrewes and Horder, again pyogenes, anginosus, and fæcalis, as well as other forms not found by them, and similarly with others of the streptococci.

The conclusion, therefore, is apparently unavoidable that, in spite of the very extended observations of Gordon, Houston, and Andrewes and Horder on the streptococci, there is still no evidence of the existence in the human subject of more than one micro-organism, Streptococcus, though this may vary as greatly in its chemical reactions in different cases as it is known to do in virulence. This view agrees with those consistently advanced by Marmorek and others.

A paper has quite recently appeared by C. O. Jensen giving an account of investigations carried out by him and his co-worker Holth on a number of micro-organisms, including streptococci, by the application of a long series of chemical and biological tests. Jensen states that Holth has examined 150 different strains of streptococci in relation to *all* the carbohydrates and polyvalent alcohols which he could obtain, and to a number of glucosides in addition. The conclusion arrived at is that culture in bouillon containing these substances, followed by *titration* of the reaction affords a valuable means of identification, differentiation, and classification of these organisms (the italics are mine). These observers regard lactose, trehalose, cellobiose, and gentobiose as of great value for the purpose of classification, and find that further differentiation is possible by the use of various polyvalent alcohols and glucosides.

Using these methods Holth and Jensen find, even in "closely similar conditions of disease," that the strains of streptococci present in different cases are different, and they ask the question whether these differences are really stable and fixed characters or are merely casual variations. Jensen states that Holth's experiments (not yet published) lead to the conclusion that the differences are actually fixed, and that the organisms concerned are distinct and well-defined forms, but possessing similar pathogenetic action.

A description of the methods which Holth has employed to prove the stability of the differences in question will be awaited with interest, since it is evident from the experiments described above that such observations need to be prolonged or the conditions of experiment sufficiently varied before definite conclusions can be safely drawn.

One conclusion of great interest follows from the observation of these authors that closely similar conditions of disease may be associated with quite different strains of streptococci. It is that the differences brought out in carbohydrate, alcohol, and glucoside containing media are in no way closely related to the virulence and pathogenetic action of the streptococci examined. Accordingly no reliable inferences as regards these most important questions can legitimately be drawn from the results obtained in the media at present under discussion. The claim of Andrewes and Horder that the results so obtained were such as to enable one the better to form a prognosis and to initiate treatment therefore appears to lack sufficient evidence.

In continuing his communication Jensen next proceeds to discuss a special streptococcal disease of horses, namely, strangles. After pointing out the fact that the course and pathology of this disease varies remarkably in different cases, he states that Holth, who has examined about 40 strains of streptococci from strangles of Danish, German, English, and Swedish origin, has by the use of a very large number of his test media definitely proved them all to be identical. And the constancy of the reactions thus found is not attributable to the residence of the organisms in the horse, since this animal harbours many other streptococci which present quite different series of reactions in the same media.

It would appear, however, that the fact that organisms so completely identical as these are found to be in the test media are admitted to vary greatly in their pathogenicity in horses only shows again how small is the assistance to be looked for from this method of identification in dealing with the practical problems of disease. It is quite possible, and indeed even probable, that the causal agent of strangles is an independent and specific organism; but this would only prove that Marmorek was correct in his conclusions as regards the streptococci, and that this organism is not a true streptococcus at all, in the usual sense, any more than is the *Streptococcus lanceolatus* of pneumonia.

Jensen makes the suggestion that when a single streptococcal form (like strangles) shows so great a constancy in the test media, it is the most probable conclusion that other streptococci also possess constant reactions. It may be pointed out that a generalisation of this character from a single instance is extremely apt to be fallacious. But a consideration of the facts adduced, namely, that on the one hand strangles presents different pathological events, the organisms being identical to the test media, while on the other hand similar conditions of disease may be occasioned by streptococci which give quite different series of reactions in these media, suggests that the reactions of true streptococci possess no fundamental relation to their pathogenicity, and present no evidence of permanence.

Although the test media have been shown to afford no evidence of the diversity of human streptococci, it is still possible that they may be made to serve a useful purpose in supplying indications as to the probable recent or habitual environment of particular strains.

A study of the very numerous and careful observations made by other workers and notably by Gordon, Houston, and Andrewes and Horder, suggests that on continued residence in a fairly uniform environment, as for example in the human mouth, the streptococcus tends to assume a moderately uniform series of reactions. But there is no satisfactory evidence as to the length of time such a process may require.

From early in 1908 our laboratory media have been prepared in a fairly uniform manner save for the natural variations in "meat extract." And it might be thought from an inspection of Table III above that the streptococci cultivated in stab agar were actually tending to a definite series of reactions such as that exhibited in 1910 by L, P, and V, and almost reached by S, M, and H, G for the time remaining refractory.

Again in Table V it is very striking how after eight weeks in inulin medium L, S, and G reach an identity which M falls short of only in the matter of clotting milk, while P appears to be moving slowly in the same direction. On returning to stab agar for three months L and P re-assume and S now assumes the reactions seen before from agar cultures.

This evidence, however, does not amount to more than a suggestion of what might prove to be the fact upon an extended trial and the use of suitably selected media.

On the other hand it is apparent that the organisms did not *always* respond very readily to changed environment in my experiments, and little or nothing is at present known of the conditions which determine such response.

As regards the investigation of the practical problems of pathogenicity and virulence it would seem more appropriate to employ media of known and definite composition instead of media based upon material so indefinite as meat extract, and to use proteins and protein derivatives as test substances rather than carbohydrates, alcohols, and glucosides.

Conclusions.

1. The reactions of any given strain of streptococcus in Gordon's media vary considerably under the conditions of ordinary laboratory cultivation, and by suitable manipulation of the culture media they can readily be made to vary very greatly.

2. The results obtained entirely oppose the view that these reactions afford a means of distinguishing fixed and definite varieties among streptococci isolated from the human subject.

3. Such differences as are observed are of a temporary and accidental character, and are not in any sense specific, though they may perhaps afford some evidence of the natural habitat or previous environment of the organisms concerned.

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HARRY MARSHALL WARD, 1854—1906.

Harry Marshall Ward, eldest son of Francis Marshall Ward, was born in Hereford, March 21, 1854, but he came of a Lincolnshire stock, settled for some time in Nottingham. From unavoidable causes he left school at 14, but afterwards continued his education by attending evening classes organised under the Science and Art Department. To that Department, as will be seen, he owed indirectly the opportunity of a useful and brilliant career. His means were small, and his earliest aim was to qualify as a science teacher. He was admitted to a course of instruction for teachers in training given by Prof. Huxley in 1874–5. Although he must have derived from it a sound insight into the principles of zoology, the subject does not seem to have had any permanent attraction for him.

In the summer of 1875 Ward came under my hands in a course of instruction in botany which I conducted with Prof. Vines in the Science Schools at South Kensington, and from this time onwards we were in intimate relations to the close of his life. I must be permitted to depart from the customary form of these notices and relate the story in my own words. It contains much of more than personal interest which could not easily be dealt with in any other way.

It was soon apparent that we had got hold of a man of exceptional ability. It must be confessed that the atmosphere was stimulating, and the conditions under which the teaching was carried on necessitated its being given at high pressure. I remember that on one occasion Ward fainted at his work, from no other cause, I think, than over-excitement. In the autumn of the same year he went for one session to Owens College, Manchester, with the object of continuing his general education. I learn that he carried off the prizes in every subject that he took up.

Impressed as I had been with Ward's capacity, I saw at the moment no outlet for it beyond that of a teacher under the Science and Art Department, for which he had become officially qualified. However, opportunity came, in the least expected way. The methods of teaching laid down by Huxley at the Science Schools were novel at the time, inasmuch as they were more demonstrational than didactic. They attracted some outside attention, and the class-rooms were a good deal frequented by visitors, who were interested in what was being done. Amongst these was a young man of private fortune, L. A. Lucas, a former pupil of Huxley's. He seems to have discovered Ward and to have watched his work. Personally, I had no hand in what followed.

In the succeeding year I was glad to avail myself of the assistance of Ward as demonstrator in a subsequent course at South Kensington, which I undertook with Prof. Vines. Some time afterwards he called on me at Kew with a strange story and to ask my advice. He had received an anonymous letter, telling him that if he would enter at the University of Cambridge, he would find a sufficient sum of money to his credit at Mortlock's Bank to pay his expenses. The acceptance of such a proposal involved a certain amount of risk, but I advised him to take it. By Vines' advice he accordingly became a candidate for and secured an open scholarship at Christ's College, where Vines himself was then a Fellow, and went into residence in October, 1876. On November 20 following, Lucas, who had planned an expedition to Africa, died on board the s.s. "Massowah" between Suakim and Jeddah at the early age of 25. He had broken down in health, and General Gordon would not allow him to proceed beyond Khartoum. He proved to have been Ward's benefactor, and had made provision in his will for the continuance of help, which was as honourable to the recipient as to the giver. The action that Lucas took was no doubt a concession to Ward's strong independence. That he took a deep interest in him is shown by a letter which he wrote to him from Cairo, November 28, 1875, when Ward was at Owens College: "Slow and sure wins the day. Work ten or even eleven hours a day. You can do that, if you keep regular hours, for an indefinite time. Always take *at least* seven hours' sleep, for the more the work the more rest required."

Ward took full advantage of his opportunities at Cambridge, and attended the teaching of Sir Michael Foster in physiology and of Prof. F. M. Balfour in comparative anatomy. The sound and fundamental conceptions which he acquired from the former manifestly influenced his work throughout life. He took a first class in botany in the Natural Science Tripos in 1879. His first published paper was the result of work in the same year in the Jodrell Laboratory at Kew. In this, which was published in the 'Proceedings of the Linnean Society,' he seriously criticised and corrected that of Vesque on the embryo-sac of Phanerogams.

As was customary with our young botanists, Ward went to Germany for a short time, for purposes of study and to strengthen his knowledge of the language. He worked at Wurzburg with Sachs, whose lectures on the physiology of plants he afterwards translated in 1887. There he continued his study of the embryo-sac in Orchideæ, as Sachs subsequently testified, "zu meiner vollsten Zufriedenheit."

Before the end of the year Ward was appointed on the recommendation of Kew to proceed to Ceylon for two years as Government Cryptogamist to investigate the leaf disease in coffee. The history of this malady is almost unique in vegetable pathology. A native fungus which had eluded scientific observation, and must therefore have maintained an inconspicuous and limited existence on some native host-plant, found a wider opportunity on the Arabian coffee plant and fell upon it as a devastating scourge. It was first detected in 1869 on a single estate; in 1873 there was probably none in the island entirely free from it. Mr. (since Sir Daniel) Morris had shown that the plants could be cleansed by dusting them with a mixture of sulphur and lime. But the remedy proved of no avail as the plants speedily became re-infected. Morris had been transferred to another appointment in the West Indies and Ward's duty was to take up the investigation. This he accomplished exhaustively. He showed that the fungus (*Hemileia vastatrix*) was one of the Uredineæ and that infection was produced by the wind-borne uredospores. Had the planters, as in Southern India, left forest belts between their plantations, the spores might have been filtered out and the disease controlled. As it was it spread like an unchecked conflagration. Ward also discovered the teleutospores; nothing has been added to our knowledge of its life-history beyond what he obtained. The result of his investigations was given in three official reports and in papers contributed in 1882 to the Linnean Society and the 'Quarterly Journal of Microscopical Science.' It was no blame to him that his work led to no practical result. The mischief admitted of no remedy. The coffee-planting industry of Ceylon was destroyed and the Oriental Bank succumbed in the general ruin. Leaf disease has now extended to every coffee-growing country in the Old World from Natal to Fiji.

In a tropical country leaves supply a substratum to a little flora of their own, consisting of organisms partly algal, partly fungal, in their affinity. Ward, who had already developed his characteristic habit of never neglecting any point incidental to a research, carefully studied them, in order both to ascertain how far their presence affected the health of the leaf itself and to work out their life-history. The outcome was three important papers. One on Meliola, an obscure genus of tropical epiphyllous fungi, belonging to the Pyrenomycetes, was published in the 'Philosophical Transactions' in 1883. Bornet's classical memoir published in 1851 had been the authority on the subject. Ward was able to fill up "large gaps in the knowledge of important details." Another paper published in the 'Quarterly Journal of Microscopical Science' in 1882 on an Asterina illuminates an allied organism. But the crown of all Ward's Ceylon work was the splendid memoir on a Tropical Epiphyllous Lichen which was published by the Linnean Society in 1883. In this he, I think, cleared up much that was obscure in the *Mycoidea parasitica* described by D. D. Cunningham. Having myself communicated the paper, I shall always remember the pleasure with which I undertook in Ward's absence to give an account of it. He solved the problem with convincing completeness; he extended Schwendener's lichen theory to a group of obscure epiphyllous organisms of which he afforded, for the first time, a rational explanation. The success with which this was accomplished placed him at once in the first rank of mycological investigators.

De Bary was the leading authority on Uredineæ; and in 1882 Ward paid a short visit to him at Strasburg to confer with him on his coffee disease work, the accuracy of which de Bary entirely confirmed. There he made the acquaintance of Elfving and completed his Meliola paper.

The outlook for Ward was now precarious. Fortunately, I found myself sitting next to Sir Henry Roscoe at a Royal Society dinner, and I suggested that Ward, as an old student of Owens College, would be a fitting recipient of a Bishop Berkeley Fellowship for original research. Principal Greenwood recorded the fact that "the very important results already achieved by Mr. Ward in Ceylon, in the domain of the higher botany, led the Senate and the Council to make this appointment." In 1883, he was appointed Assistant Lecturer and Demonstrator in Botany, and, on the same testimony, "abundantly justified his election." It was a peculiar pleasure to him to relieve the veteran Prof. Williamson by taking entire charge of Vegetable Physiology and Histology. His position was, in the same year, made secure by his election to a Fellowship at Christ's College, and he married the eldest daughter of the late Francis Kingdon, of Exeter, who was a connection of Clifford the mathematician.

The passion for research now completely possessed Ward and never left him for the rest of his life. He published papers which added much to our knowledge of the Saprolegnieæ a group of fungi of aquatic habit, partly saprophytic and partly parasitic. It is interesting to note that he was particularly attracted by the mode in which the hyphæ attack the tissues on which they prey. This was a matter on which he subsequently threw an entirely new light. He made the interesting discovery of an aquatic Myxomycete, such a mode of existence being hitherto unknown in the group, and worked out its life history. But his mind had now become definitely fixed on the problems presented by plant diseases, and they remained the principal occupation of his life. In their widest sense these resolve themselves into a consideration of the mode in which one organism obtains its nutriment at the expense of another. This ranges from a complete destruction of the host by the parasite to a harmless and even advantageous symbiosis. He was thus naturally led to an exhaustive study of the literature of the Schizomycetes, and contributed an article on the group in 1886 to the 'Encyclopædia Britannica,' which, for the time at any rate, gives the best account of it, certainly in English, and probably in any other language. When he supplemented this in 1902 by the article on Bacteriology, it was largely to give an account of his own important discoveries. In the earlier one, he had pointed out the difficulties of a natural classification of Schizomycetes due to their pleomorphism, which Lankester had demonstrated in 1873. He returned to the subject in an article in the 'Quarterly Journal of Microscopical Science' in 1892. It may be noted that, in his British Association address at Toronto, he took occasion to put in their proper relation the work of Cohn and of his pupil Koch.

In 1885, the Regius Professorship of Botany at Glasgow was vacant by the transference of Prof. Balfour to Oxford. Ward was a candidate with the warm support of his fellow-botanists. It was thought that his Colonial services would weigh with the Government; but other influences were at work in favour of another candidate, whom, however, the University refused to accept. A deadlock ensued, which was only solved by the Government finally refusing to appoint either candidate. This was a great disappointment to Ward, which was in some degree mitigated by his appointment to the new Chair of Botany in the Forestry Branch of the Royal Indian Engineering College, Coopers Hill. The utilitarian atmosphere in which he found himself was not very congenial to him. But he had at any rate at last some sort of adequate position and a laboratory to work in, and here he remained—not, I think, unhappily—for ten years. He was, as he had been at Manchester, a successful teacher, and had the gift of interesting his pupils, whom he used to bring weekly to Kew during the summer months to visit the Arboretum. In point of research, this was the period of much of his most brilliant work.

The study of Uredineæ occupied Ward at intervals during his life. The reproductive organs are pleomorphic, and it is no easy task to ascertain with certainty those that belong to the same life-history. In a paper on *Entyloma Ranunculi*, published in the 'Phil. Trans.' in 1887, Ward for the first time traced the germination of the conidia of an Entyloma, and confirmed Winter's suggestion that they were not an independent organism, but actually belonged to it. Incidentally he discussed the conditions which are favourable to the invasion of a host by a parasitic fungus. This raised the question of immunity, to which at intervals he repeatedly returned.

About the same time he published in the 'Quarterly Journal of Microscopical Science' the results of an investigation undertaken for the Science and Art Department on the mode of infection of the potato plant by *Phytophthora infestans,* which produces the potato disease. It was not easy to add anything to the classical work of de Bary, but it was ascertained that "the development of the zoospores is delayed or even arrested by direct daylight," and Ward's attention was attracted to the problem, which he afterwards solved, of how the hyphæ erode the cell-wall.

The solution was given in 1888 in a paper in the 'Annals of Botany,' "On a Lily Disease," which has now become classical. He discusses the fungus which produces it, and shows that the tips of the hyphæ secrete a cellulose-dissolving ferment which enables them to pierce the cell-walls of the host. This ferment has since been described as cytase. He shows that its production would determine the passage from a merely saprophytic to a parasitic habit, and makes the suggestion that an organism might be educated to pass from one to the other.

An admirable research (1887) was on the formation of the yellow dye obtained from "Persian berries" (*Rhamnus infectorius*). A dyer had found that uninjured berries afforded a poorer colouring liquor than crushed. Gellatly had found, in 1851, that they contained a glucoside, xanthorhamnin, which sulphuric acid broke up into rhamnetin and grape-sugar. The problem was to localise the ferment which did the work. Ward obtained the unexpected result that it was confined to the raphe of the seed.

As early as 1883 Ward had attacked a problem which he pursued at intervals for some years, and which was fraught with consequences wholly unforeseen at the time. It had long been known that leguminous plants almost invariably carried tubercular swellings on their roots. The opinion had gradually gained ground that they were due to the action of a parasite. Bacteria-like corpuscles had been found in the cells of the tubercle, and it was assumed that they had played some part in exciting the growth of the latter. "No one had as yet succeeded in infecting the roots and in producing the tubercles artificially." Ward described, in a paper in the 'Phil. Trans.' in 1887, how he had accomplished this. He showed, in fact, that a definite organism invades the roots from the soil, and finds its access by the root-hairs.

Lawes and Gilbert had long ago proved that the higher plants are incapable of assimilating free nitrogen. Hellriegel and Wilfarth had, however, shown in 1886 that leguminous plants carry away more nitrogen from the soil than could be accounted for. This Ward confirmed by his own pot-experiments and satisfied himself that the excess could only be derived from the free nitrogen of the air. Hellriegel further concluded that the tubercles played an essential part in the process. Ward had no doubt that the bacteroids were the channel of supply. But he failed to get any proof that they could assimilate free nitrogen outside the plant. He suggested that their symbiosis might be an essential condition, and was obliged finally to leave it an open question whether the cells of the tubercles or the bacteroids were the active agents in nitrogen assimilation. He had already stated in 1887 that it is very probable that the bacteroids "may be of extreme importance in agriculture." But he was never satisfied with anything short of the strictest proof.

In 1890 Ward was invited to deliver the Croonian Lecture. He chose for his subject the relation between host and parasite in plant disease. He defined disease in its most generalised form as "the outcome of a want of balance in the struggle for existence." But the particular problem to which he addressed himself was the way in which the balance is turned when one organism is invaded by another. This is the most common type of disease in plants and a not infrequent one in animals. The first result reached was identical with that of Pasteur for the latter; the normal organism is intrinsically resistant to disease. It is an immediate inference that natural selection would make it so. Ward then discusses very clearly the physiological conditions of susceptibility, which he shows to be a deviation from the normal. He had already indicated this in the case of Entyloma. The epidemic phase is reached when the environment is unfavourable to the host but not so or even favourable to the parasite. He then attacks the more obscure case where there is no obvious susceptibility. This, he finds, resolves itself into a mere case of the struggle for existence: "a struggle between the hypha of the fungus and the cells of the host." It is more subtle in its operation but of the same order of ruthlessness as the ravages of a carnivore. Ward's account of the struggle is almost dramatic. The cellulose "outworks" are first broken down, as he had previously shown, by a secreted ferment. The "real tug of war" comes when the hypha is face to face with the ectoplasm. Its resistance is at once overcome by flooding it with a poison, probably oxalic acid.

War with attack and defence is a product of evolution. How did it come about in this particular case? Ward convincingly traces out the whole process. The normal plant obtains its food from inorganic material. But when opportunity offers it easily lapses into a condition in which it takes the material for metabolism ready made from the decay of others and becomes saprophytic. Ward shows that it is only a step to the attack on the living, and for the saprophyte to become a parasite, and he further shows that it can be readily educated to be so. He does not hesitate to suggest that the function of conidia in the complicated cycle of fungal reproduction is to form the cellulose-dissolving ferment. But now and again the host does not succumb to its invader. A truce is sometimes called in the struggle, and host and parasite are content to live together in a mutually advantageous symbiosis or commensalism.

Three years earlier, in 1887, Ward's attention had been drawn by a happy accident to the physiological aspect of symbiosis, and it never ceased to occupy his mind. It was well known that ginger beer was made in villages in stone bottles. The fermentation was effected by the so-called "ginger beer plant" which was passed on from family to family, but nothing was known as to how or where it originated. It seemed to have some analogy with the Kephir of the Caucasus. A specimen was sent to me from the Eastern Counties, and it stood for some time in the sun in my study. I noticed the vigorous growth accompanied by a copious evolution of gas. Ward coming to see me one day, I handed it over to him as a problem worth his attention. At the same time Prof. Bayley Balfour had examined it and concluded that it was a mixture of a yeast and a bacterium. Its study involved Ward in a very laborious research which occupied him for some years, and of which the results were published in the 'Phil. Trans.' in 1892. It proved to be a mixture of very various organisms, every one of which Ward exhaustively studied. This required not less than 2000 separate cultures. The essential components proved to be, as Balfour had suggested, a yeast derived from the sugar and a bacterium from the ginger. Both were anaërobic; the yeast fermented cane-sugar with the copious production of carbon dioxide but little alcohol; the bacterium also produced carbon dioxide, even in a vacuum tube.

The action of the two components studied separately proved to be not the same as when they worked in concert. This was conspicuously the case with the evolution of carbon dioxide, which proceeded with such violence as to make the research attended with considerable danger. It is known that the action of ferments may be checked by the inhibition of the products formed. Ward pointed out that while the use of these might be advantageous to the bacterium, their consequent removal might be equally so to the yeast. This established the important principle of symbiotic fermentation and gave it a rational explanation. On the morphological side Ward showed that the ginger-beer plant is comparable to a gelatinous lichen, and, having resolved it into its constituents, successfully reconstituted it.

The new conception threw a flood of light on many obscure points in fermentation generally, and it is not surprising that Ward's work at once attracted the attention of the brewing industry. It led him to an even more fertile suggestion, that of metabiosis. It was known that the finest wine is sometimes produced from mouldy grapes. He regarded this as a case of one organism preparing the way for another. He returned to the subject in a lecture given at the British Association at Dover in 1899 and pointed out that in the Japanese manufacture of Saké, an Aspergillus prepares the way for the yeast. He also showed that metabiosis played an important part in nitrification.

Fungi cannot draw their nutriment from solid materials without first profoundly modifying them. They accomplish a large part of their digestion, so to speak, externally to themselves. This constantly occupied Ward's mind. He insisted on the part played in the process by ferments. The hyphæ of Stereum ('Phil. Trans.,' 1898) delignify the walls of the wood elements of Æsculus layer by layer, and then consume the swollen cellulose. He failed, however, to isolate the ferment which does the work. Nor was he more fortunate with the little known fungus Onygena, which grows on horn, hoofs and hair, setting free ammonia as a final product ('Phil. Trans.,' 1899). That there must be some hydrolysis of keratin can hardly be doubted, for Ward established the remarkable fact that the walls of the hyphæ contain no cellulose, but are composed of chitin. Onygena has, in fact, abandoned a plant for an animal nutrition. This would place the germination of the species at a great disadvantage. But he found that this difficulty was overcome by the spores which had been licked from the skin germinating in the gastric juice of the animal's stomach, and, when voided in the excreta, infecting a new host by accidental contact. In the case of both Stereum and Onygena he accomplished for the first time the difficult task of tracing their life history from spore to fructification.

Ward had prepared himself for the study of bacteria, and in the nineties he undertook, with Prof. Percy Frankland, a prolonged research on behalf of the Royal Society as to the conditions of their occurrence in potable water. The reports of the results fill a thick volume, and the amount of work involved is almost incredible. The bacteriology was entirely due to Ward.

That bacteria are not an inevitable element in potable water is proved by their absence from that of deep springs. They are arrested by filtration through the earth's crust. In any river system they are comparatively fewer towards the watershed, and more frequent towards the mouth. The obvious conclusion is that they are derived from the drainage of the land. As it is known that the bacteria of cholera and typhoid are water-borne, it becomes a problem of vital importance to ascertain if river water is a possible means of distributing these diseases. Ward set to work to ascertain: (i) What was the actual bacterial flora of Thames water; (ii) if this included any pathogenic organisms; (iii) if not, what became of them?

The labour required by the first two branches of the enquiry was enormous; he identified and cultivated some eighty species; the resulting answer to the second was happily in the negative.

As to the third, two facts were known. First, that river water, if stored, largely cleared itself of bacteria by mere subsidence; secondly, that Downes and Blunt, in a classical paper communicated to the Royal Society in 1877, had shown that exposure to direct sunlight is fatal to bacteria in a fluid medium. Ward showed that subsidence could not be entirely relied on, as the sediment might easily become the source of re-infection. The effect of sunlight required more critical examination.

It was known that the spores of anthrax were liable to be washed into rivers. Ward determined to study this as the most extreme type of pathogenic infection. As it is undoubtedly the most deadly micro-organism known, and Ward proposed to deal with it on a large scale, it implied no small degree of courage. He found that the spores of anthrax were effectually killed by a few hours' exposure to even the reflected light of a low winter sun. It was clear that this was due to the direct action of the light and not to any heating effect, apart from the fact that they will tolerate boiling for a few minutes. It was further shown that there was no foundation for the theory of Roux and Duclaux that death was due to poisoning by products of oxidation of the food-medium. Proof of this, indeed, was hardly required, for Pasteur had shown that the bacteria floating in the atmosphere are mostly dead. Were it not so, no surgical operation would be possible. To the bactericidal effect of sunlight is equally to be attributed the absence of bacteria from the High Alps.

The next point was to ascertain to what rays the effect was due. The spores of anthrax are so minute that, when mixed in large numbers with gelatine, they do not affect its transparency. A plate of glass coated with the mixture is at first clear, but ceases to be so if kept in the dark, owing to the germination of the spores. Ward found, in fact, that a photograph could be printed with it, the darkening being the reverse of that of a silver plate. After experiments with coloured screens he completely solved the problem in 1893, with the aid of apparatus supplied by Sir Oliver Lodge and some advice from Sir Gabriel Stokes; by photographing the spectrum on such a plate. It was at once seen that the destructive effect was due to rays of high refrangibility, and, what was extremely important, extended to, and found its maximum in, the ultra-violet. The same results were obtained with the typhoid bacillus. He made the suggestion that the arc light might be used for the disinfection of hospitals and railway carriages.

Comparatively little was known of the life history of any Schizomycete. Ward therefore made a detailed and exhaustive study of that of *Bacillus ramosus*, the *Wurzel bacillus* of German authors, which is common in Thames water, and bears a superficial resemblance to the anthrax bacillus, but is innocuous. It proved convenient for study, as it ran through its entire life history in from thirty to sixty hours at ordinary temperature. It forms long filaments, the growth of which Ward was able to measure under the microscope with great precision. On plotting out his measurements he obtained a regular curve, from which he found that, under constant conditions, the filament doubled itself in equal times. This he called "the law of doubling." It is the same as the so-called "law of compound interest," and leads to the expression of the growing quantity as an exponential function of the time, so that the time is proportional to the logarithm of that quantity. This relation has, of course, long been familiar in chemical reactions, but, as far as I know, Ward was the first to detect it in any vital process in a plant. This, which was in 1895, has, I think, been overlooked. Stefanowska has since, in 1904, obtained a logarithmic curve for the early period of the growth of maize, which doubles its weight every ten days, and the subject has since been pursued by Chodat and others.

In speculating on the cause of the destructive action of light on bacteria, Ward adopted the view of his friend Elfving, that it inhibited metabolic processes necessary to nutrition. He suggests that the "constructed metabolites" at the moment of assimilation are in a highly unstable condition, and liable to destruction by oxidation promoted by light. He points to the fact that plant structures are frequently provided with colour screens, which would cut off the blue-violet rays and check their action in promoting the rapid oxidation of reserve materials, and he quotes the suggestion of Elfving that chlorophyll itself may serve as such a screen against "destructive metabolic action in synthesis." Ward seems to have attributed little importance to the fact that substantially the same view had long before been put forward by Pringsheim, though received with little favour. His own view that when red and orange predominate in the screens their effect is protective, has since afforded a probable explanation of the coloration of young foliage, especially in the tropics.

It can hardly be doubted that the upshot of Ward's laborious investigations has had a powerful influence in deciding the policy of the future water supply of London. If we hear nothing now of obtaining it from Wales, it is because we know that even polluted flood-water if exposed in large reservoirs will rid itself of its bacterial contamination, partly, as was known already, by subsidence, but most effectually, as shown by Ward, by the destruction of its most deleterious constituents by the direct action of sunlight.

In 1895, Ward was called to the Chair of Botany at Cambridge. He was supported by a distinguished body of fellow-workers, and developed a flourishing school, in which every branch of the science found its scope. The University erected for it an institute which is probably the best equipped in the country, and in March, 1904, I had the pleasure of seeing Ward receive the King and Queen at its inauguration.

During the later years of Ward's life he returned to the study of the

Uredineæ. The scourge of wheat perhaps from the dawn of agriculture has been "Rust,"

> "Ut mala culmos esset rubigo . . . . intereunt segetes";

and the loss inflicted by it throughout the world is probably not calculable. But the history of the Ceylon coffee disease is only too patent an instance of the injury a uredine can effect.

Eriksson, the most recent authority on the subject, had found himself quite unable to account for sudden outbursts of rust which it did not seem possible to attribute to the result of infection. In 1897 he launched his celebrated theory of the Mycoplasm. He supposed that a cereal subject to rust was permanently diseased and always had been; that the protoplasm of the Uredo-parasite and of the cereal, though discrete, were intermingled and were continuously propagated together; but that while that of the latter was continuously active, that of the former might be latent till called into activity by conditions which favoured it. Ward discussed the theory in his British Association address at Toronto, and was evidently a good deal impressed with it, but nothing short of actual demonstration ever convinced him; and when he proceeded to investigate the actual histological facts on which the theory rested he promptly exploded it.

It is interesting to note that Ward, as I know from correspondence at the time, had himself been embarrassed in investigating the Ceylon coffee disease by the same kind of appearance which had misled Eriksson. It is due to an optical fallacy. When the hypha of a uredine attacks a cell it is unable to perforate it with its whole diameter. It effects it, however, with a reduced and slender filament; this expands again after perforation into a rounded body, the haustorium. In a tangential section the perforating filament cannot be distinguished, and the haustorium looks like an independent body immersed in the cell-protoplasm and with no external connection. It requires a fortunate normal section to reveal what has really taken place. Ward was accordingly able, in a paper in the 'Phil. Trans.' in 1903, to conclusively dispose of the mycoplasm. This cleared the ground of an untenable hypothesis. The complicated nature of the problem which still presented itself for investigation can only be briefly indicated. Sir Joseph Banks, whose scientific instinct was sound but curiously inarticulate, had pointed out that the spores entered the stomata, and warned farmers against using rusted litter. Henslow, one of Ward's predecessors in the Cambridge chair, had been confirmed by Tulasne in showing that the uredo- and puccinia-spores (of the barberry) belonged to the same fungus. De Bary traced the germination of the spores and the mode in which the hyphæ invaded the host; the fundamental fact, which he observed but did not explain, was that the germinal filament, after growing for a time superficially, bent down to enter the tissues of its host. Pfeffer in 1883 discovered chemotaxis, the directive action of chemical substances on the movement of mobile organisms. De Bary had previously hinted that the hypha might be attracted by some chemical ingredient of the host plant. Myoshi, a pupil of Pfeffer's, showed finally in

1894 that if a plant were injected by a chemotropic substance a fungus-hypha not ordinarily parasitic might be made to behave as such and attack it.

In such circumstances it might seem that the host was not merely incapable of resisting invasion by the parasite but actually invited its attack. Nature is, however, not easily baffled in the struggle for existence. Attack provokes new methods of defence. Ward soon found himself face to face with "problems of great complexity," and these occupied the closing years of his life.

It had been ascertained in fact that the rust fungus is not, as was at first supposed, a single organism, but comprises, according to Eriksson, thirteen distinct species, each with physiological varieties, and that those which are destructive to some grasses and cereals, are incapable of attacking others. This necessitated a scrutiny of the nature of grass-immunity. In a paper communicated to the Cambridge Philosophical Society in 1902, Ward announced a conclusion which was as important as it was unexpected. He had more and more made use of the graphical method for presenting to the eye at a glance the result of a mass of separate observations. In this case he uses it with striking effect. He shows conclusively, as far as rust in brome-grasses is concerned, that: "The capacity for infection, or for resistance to infection, is independent of the anatomical structure of the leaf, and must depend on some other internal factor or factors in the plant."

Finally, he is led to the conclusion that "it is in the domain of the invisible biological properties of the living cell that we must expect the phenomena to reside." He pointed out the probability that light would be thrown on this from the action of chemotaxis, on the one hand, and from that of toxins and antitoxins in animal organisms on the other. This is a most fertile conception, which would, however, have required a good deal of verification, and this, unhappily, he did not live to attempt. But with characteristic ingenuity he pointed out the analogy between the infective capacity of uredospores and the prepotency of pollen, which had previously engaged the attention of Darwin. In a paper published in the following year in the Berlin 'Annales Mycologici,' he announced a no less significant result. With his usual thoroughness in research he had cultivated side by side at Cambridge more than two hundred species and varieties of Bromus, and had watched the degree to which they were infected by rust under identical conditions. He found that though in the brome-grasses the rust peculiar to them is specifically identical its *forms* are highly specialised. The form which attacks the species of one group will not attack those of another. Host and parasite are mutually "attuned." He termed this "adaptive parasitism." This raised the problem, which had first occurred to him in Ceylon, of how a parasite adapted to species of "one circle of alliance" can pass to those of another. Occasionally it happens that a uredo-form will infect a species where it ordinarily fails. In such a case "its uredospore progeny will thenceforth readily infect that species."

Ward regarded this as a case of education. Working on this principle, he succeeded by growing the parasite *successively* on a series of allied species which were imperfectly resistant, to ultimately educate it to attack a species hitherto immune. He called these "*bridgeing species.*" He established, in fact, a complete parallelism between the behaviour of rust-fungi and that of pathogenic organisms in animals.

In the midst of this far-reaching research his health began to fail. In 1904 he had been appointed by the Council to represent the Royal Society at the International Congress of Botany held at Vienna in June of the following year. This he attended, though more seriously ill than he was aware of. On his way back he spent three weeks for treatment at Carlsbad, but receiving no benefit, he went, on the advice of Dr. Krause, to Dr. von Noorden's Klinik at Sachsenhausen (Frankfort). Nothing could be done for him, and he was advised to return home by easy stages. After a period of progressive and extreme weakness, borne with unflinching courage, the end came somewhat suddenly at Torquay on August 26, 1906. He was buried at Cambridge in St. Giles's Cemetery on September 3.

From 1880, the year following his degree, Ward never ceased for a quarter of a century to pour out a continuous stream of original work. This alone would be a remarkable performance, had he done nothing else. But he was constantly engaged in teaching work, and he acted as examiner in the Universities of London and Edinburgh. With no less conscientiousness he complied with the demands which the scientific world makes on its members; he served on the Councils of the Royal (1895) and Linnean (1887) Societies; he was President of the Botanical Section of the British Association at Toronto in 1897, and of the Cambridge Philosophical Society in 1904. Beyond all this he found time to give addresses with unfailing freshness of insight; a lecture at the Royal Institution on April 27, 1894, on the "Action of Light on Bacteria and Fungi" was a notable performance; he wrote numerous articles of a more popular kind, and he produced a number of excellent manuals for students on subjects connected with forests, agricultural and pathological botany. Activity so strenuous almost exceeds the limits of human possibility.

Under the influence of Sachs, Ward might have become a distinguished morphologist. But his work in Ceylon led him into a field of research from which he never deviated. A survey of his performance as a whole, such as I have attempted, has a scientific interest of it own. His research was not haphazard. A continuous and developing thread of thought runs through it all. The fundamental problem was the transference of the nutrition of one organism to the service of another. Of this, in Ceylon, Ward found himself confronted with two extreme types, and of both he made an exhaustive study. In Hemileia it was ruthless parasitism; in Strigula advantageous commensalism. Our Foreign Fellow Bornet put Schwendener's theory on a firm foundation when he effected the synthesis of a lichen; Ward, in another group, did the same thing for the ginger-beer plant. In such cases the partnership is beneficial. The problem is to trace the process by which one partner gets the upper hand and becomes merely predatory. Ward inherited a strong taste for music, though I believe he never cultivated it. A musical simile may not inappropriately be applied to his work. In its whole it presents itself to me as a symphony in which the education of protoplasm is a recurring *leit-motiv.*

A few words must be said as to his personal characteristics. He had all the qualifications for the kind of research to which he devoted himself. He was singularly dexterous and skilful in manipulation. He was a refined and accomplished draughtsman, and was therefore able to do himself justice by illustration. He was rigorous in demanding exhaustive proof. This almost deteriorated into a defect. He would pursue every side issue which presented itself in a research, and was quite content if it led to nothing. He would say in such a case: "I will not leave a stone unturned." He was apt, too, I think, to attack a problem in too generalised a form. In his nitrogen work it always seemed to me that he wasted energy on remote possibilities, when a clean-cut line of attack would have served him better. But his mind worked in that way, and he could not help himself. It was, I think, one of the most fertile in suggestion that I ever came across. In later years, in conversation especially, thought seemed to come quicker than words to express it. In this respect he reminded one of Lord Kelvin. In such a predicament he would simply remain silent, and slowly move his head. This habit, I think, explains the reputation of being "mysterious" which he seems to have acquired latterly at Cambridge.

He was not without the honour at home which he deserved, apart from the affection of his friends, and had he lived would doubtless have received it from abroad. He was elected F.R.S. in 1888, and received the Royal Medal in 1893. He was elected an Honorary Fellow of Christ's College in 1897, and received an Honorary D.Sc. from the Victoria University in 1902.

Botanical science could ill spare his loss at the early age of 52. But it may be grateful for 25 years of illuminating achievement. It might have been hoped that another quarter of a century would be allotted to one so gifted. But if the "inexorabile fatum" decreed otherwise, he is at least to be numbered amongst those of whom it may be said

> "Felix qui potuit rerum cognoscere causas."

W. T. T.-D

HARRY GOVIER SEELEY, 1839—1909.

Harry Govier Seeley was the son of Richard Hovell Seeley by his second wife Mary Govier, and was educated at a private school in London. He was born in London on February 18, 1839, and began early to take an interest in Natural History. While still a boy he attended the lectures of Owen, Edward Forbes, Brayley, and others. He also took advantage of the opportunities for study afforded by the British Museum. At the age of 20 he attracted the notice of the Rev. Adam Sedgwick, Woodwardian Professor of Geology at Cambridge, who appointed him to be his assistant. Until 1871, Seeley acted as his deputy in lecturing and teaching when the infirmities of age necessitated his rest from duties, and during the whole period Seeley was actively engaged in both curatorial work and researches in the Woodwardian Museum. In 1872 he returned to London, and was for some time engaged in lecturing and literary work. In 1876 he became Professor of Geography in King's College and Professor of Geology and Geography in Queen's College. In 1881 he was appointed Dean of Queen's College. In 1891 he also became Lecturer on Geology and Mineralogy in the Royal Indian Engineering College, Coopers Hill, and held this office until the closing of the College in 1906. In 1896 he entered on the duties of the combined Professorships of Geology, Mineralogy, and Geography in King's College, where he designed and equipped new laboratories, and worked until his last illness at the close of 1908.

While occupied with these more serious professional duties, Prof. Seeley also devoted much time to the popularisation of geological science. During the decade 1880–90 he lectured under the auspices of the London Society for the Extension of University Teaching, and for a period of twenty-five years he conducted the London Geological Field Class, which made a series of weekly excursions each summer to study field geology under his guidance. He was also a well-known and much-appreciated lecturer in connection with the Gilchrist Educational Trust.

From his earliest youth Prof. Seeley was imbued with the spirit of original research, and his appointment as assistant to the Woodwardian Professor at Cambridge gave him ample opportunities for following his inclinations. While accompanying Prof. Sedgwick in the field, and while preparing the lectures which he was so often called upon to deliver, he made many new observations on the rocks and fossils of Cambridgeshire and the neighbouring counties, which he published in a series of papers between 1859 and 1868. His researches on the Red Chalk and the Ampthill Clay were especially important. He soon became most interested, however, in the fragmentary reptilian bones from the Cambridge Greensand, which were then being discovered in large numbers in the phosphate diggings, and had never been closely studied. He arranged the collection in the Woodwardian Museum, and gradually extended his work to other groups of Vertebrate fossils until he became an accomplished palæontologist, with an intimate knowledge of the Vertebrate skeleton. In 1869 the Cambridge Press published his 'Index to the Fossil Remains of Aves, Ornithosauria, and Reptilia from the Secondary System of Strata arranged in the Woodwardian Museum,' and in the following year the same Press issued his well-known volume on 'The Ornithosauria: An Elementary Study of the Bones of Pterodactyles, made from Fossil Remains found in the Cambridge Upper Greensand.' The latter was in many respects a pioneer work, describing unpromising materials but with important results, and if it now appears to have emphasised too strongly the relationships of Pterodactyles to Birds, it was at least stimulating and led to useful discussion. During subsequent years Prof. Seeley often recurred to the same subject, collecting facts as new discoveries accumulated, until in 1901 he published his small general work, entitled 'Dragons of the Air: An Account of Extinct Flying Reptiles,' in which he summarised his latest views, with restored sketches of some of the animals described. Among other features, he was the first to recognise the curiously bird-like shape of the brain in Pterodactyles, and to point out that at least one of the Cretaceous genera was toothless.

Prof. Seeley's studies of Pterodactyles led him to recognise in 1870 some very large vertebræ, of light construction, from the Wealden formation, which he described as representing "Ornithopsis, a gigantic animal of the Pterodactyle kind." One of these bones had previously been determined by Mantell and Owen as the tympanic (or quadrate) of Iguanodon. Through the subsequent discoveries of the Rev. W. D. Fox in the Wealden of the Isle of Wight, it became clear that such vertebræ belonged to a large Dinosaur; and the group it represents is now well known through the finding of nearly complete skeletons of Diplodocus and Brontosaurus in the Upper Jurassic formations of North America. Prof. Seeley was always interested in the Dinosauria, and his determination of their fragmentary remains from the Cambridge Greensand and from the Gosau formation of Austria may be specially mentioned. In 1879 he read to the Scientific Club in Vienna a paper on Dinosaurs in general, which was afterwards published in the 'Popular Science Review,' and in 1887 he discussed before the British Association the classification of the Dinosauria, proposing that they should be divided into the two orders of Saurischia and Ornithischia.

Prof. Seeley also made an exhaustive study of the marine reptiles, and was particularly interested in comparing those of the Cretaceous and Upper Jurassic with the better-preserved specimens from the Lias. He distinguished and described the Ichthyosaurian genus Ophthalmosaurus and the Plesiosaurian genera Muræenosaurus and Cryptocleidus from the Oxford Clay, and an examination of the Leeds collection enabled him to discover the characters and variations of the shoulder-girdle in these reptiles. He described in detail the structure of a well-preserved Ichthyosaurian skull from the Upper Lias, and made some of the earliest observations on the supposed viviparous reproduction of Ichthyosauria. He also described a small reptile, Neusticosaurus, from the Trias of Germany, which appeared to be intermediate between primitive land-reptiles and the Plesiosauria.

For many years Prof. Seeley had followed closely the researches of Sir Richard Owen on the Karoo reptiles from South Africa, which exhibited so remarkable an approach to the Mammalia, and, when the veteran palæontologist ceased work, he decided to continue it. In 1889, with the aid of the Royal Society's Government Grant Committee, he examined the collections of Anomodont reptiles in St. Petersburg and Moscow, and subsequently visited Cape Colony to make further collections. He traversed the Karoo with Mr. Thomas Bain, and succeeded in discovering the nearly complete skeletons of Pariasaurus and Cynognathus, and numerous other specimens, which he afterwards described and presented to the British Museum (Natural History). He also obtained important specimens for study from Mr. Alfred Brown, Dr. Atherstone, and Dr. Kannemeyer, who continued to correspond and provide material for his researches. Most of the results of this work were published in the 'Philosophical Transactions' between the years 1889 and 1895, and in the journals of the Geological and Zoological Societies during later years. The Pariasaurians were shown to make as close an approach to the Labyrinthodonts as the Theriodonts to the Mammalia; numerous new types were made known, and their osteology was described in detail, while important general results followed, with suggestions for a more exact classification of the primitive groups represented. These exhaustive researches led Prof. Seeley to recognise an Anomodont reptile from the Bunter Sandstone near Basle, Switzerland, which he described under the name of *Aristodesmus ruetimeyeri*.

After his early experiences in interpreting the reptilian bones from the Cambridge Greensand, rare fragmentary fossils always had a special fascination for Prof. Seeley, and a large proportion of his smaller papers deal with such specimens. Among these may be specially mentioned his descriptions of the bones of birds from the Cambridge Greensand (1876) and of two Mammalian bones from the Stonesfield Slate (1879). He pointed out the resemblances of the Cretaceous bird-bones to those of the existing Colymbus, and he regarded the Stonesfield limb-bones as belonging to a generalised Marsupial.

Prof. Seeley was a Fellow of the Linnean, Geological, Zoological, and Royal Geographical Societies, and was elected a Fellow of the Royal Society in 1879. He was awarded the Lyell Medal of the Geological Society in 1885. He was elected a Foreign Member of the Philadelphia Academy in 1878, of the Imperial Geological Institute of Vienna in 1880, of the Imperial Society of Naturalists of Moscow in 1889, of the Senckenberg Natural History Society of Frankfurt in 1895, a Corresponding Member of the Imperial Academy of Sciences, St. Petersburg, in 1902, and a Fellow of King's College, London, in 1905. He was also a member of the Athenæum Club.

A. S. W.

ROBERT KOCH, 1843—1910.

PROF. KOCH was one of the great discoverers of medicine. His researches have exercised a profound influence not only upon the development of medical science, but also upon the welfare of mankind.

Born in 1843 at Klausthal, and educated at the Gymnasium, he studied medicine in Göttingen from 1862 to 1866. After a short period as assistant at the hospital in Hamburg, he commenced practice in Langenhagen, Hanover. In 1867 he removed to Rackwitz, in Posen, where, in addition to carrying on a country practice, he found time to study for, and to take a degree in physical science. In 1872 he became district surgeon in Wollstein. It was whilst at Wollstein that Koch's attention was first seriously turned to the interpretation of infectious diseases. The study of the work of Pasteur and his pupils on fermentation and putrefaction, and of Lister on the antiseptic treatment of wounds, led him to the conclusion that the etiology of infection was not to be found in miasmata from the soil, as commonly entertained at this time, but much more probably, in the entrance into the tissues of microbes, and their multiplication therein.

At the time Koch commenced to investigate infectious disease, bacteriology had become differentiated as a department of scientific enquiry, but the methods proper to the new science were not developed, and, although a number of cardinal facts had been brought to light, knowledge on the subject was chaotic, and advance temporarily checked. Diseases of man and animals presented unlimited problems, but the means to attack them were lacking. The means which led to the next important advances were supplied by Robert Koch, who possessed that rare combination of intellectual qualities which enabled him, not only to see what was the next question to ask of Nature in order to advance one step further, but also to devise experimental methods which ensured an answer to this question. In this last faculty Koch was pre-eminent, and the methods of this youngest of the sciences are to a large extent the methods of Robert Koch.

Two of his earliest papers—that on the etiology of anthrax, founded on the life-history of *Bacillus anthracis* published in 1876, and that on experiments on the etiology of wound infections, published in 1878, written when district surgeon at Wollstein—have become classics. This work was carried out in addition to the duties of a practitioner of medicine, and without the assistance of any laboratory equipment beyond a good microscope. Pollender and Davaine had seen the anthrax bacillus twenty years earlier in the blood of infected animals, and in 1863 the latter had shown that the blood containing the bacilli was capable of infecting animals, if inoculated into them. That these bacilli were in reality the cause of the disease was, however, controverted. Koch reasoned that as the disease remained attached to certain pastures, if the anthrax bacilli were the living virus, they ought to grow outside the body as well as inside. He succeeded in cultivating many successive generations of them in broth, and also watched their growth upon a hot stage. He discovered that they formed spores when grown outside the body or when blood containing them was allowed to dry; determined the greatly increased resistance of the spores to physical and chemical agents; and showed that as long, and only as long, as the broth or dried material contained bacilli or spores capable of propagating themselves, these remained infective for animals.

The importance of the anthrax work can hardly be over-estimated. It afforded for the first time convincing proof of the causal relation of a particular bacillus to a particular disease. Owing to the unmistakable character of the bacillus, and its presence in large numbers in the blood of infected animals, its study could be profitably undertaken with the means available.

Koch was unremitting in his efforts to improve his microscopical technique, and in the same year published a paper on the investigation, preservation, and photographing of bacteria, in which an account of the preparation and staining of dry films is given. The method described is very much that still in daily use. The paper is accompanied by photomicrographs of bacteria, the excellence of which is rarely equalled at the present day. Koch pointed out that he had persevered in this work because he was obsessed with the idea that the hitherto conflicting results of investigations on the causation of infective diseases had their foundation in the incompleteness of the methods used.

Koch's interest in traumatic infectious diseases seems to have been stimulated by the disasters due to these causes amongst the wounded in the Franco-Prussian war. The results of Lister's antiseptic methods had demonstrated that means directed against the infection of wounds with microbes obviated these diseases. Micrococci and bacteria had frequently been found in pus, diphtheritic ulceration, in the tissues at the edge of advancing erysipelas, and in pyæmic deposits. Micro-organisms had also been discovered in the blood in relapsing fever and puerperal fever. Further, Coze and Feltz and Davaine had inoculated rabbits with the blood of patients dead of puerperal fever, and had succeeded in carrying on the infection through successive generations of these animals. Nevertheless, the evidence that a particular organism was the cause of a particular disease was far from conclusive. Many observers concluded that bacteria were universally present in the normal body. Others failed to find any organisms in obviously septic conditions. There was no practicable means of separating one coccus from another coccus, and bacteria of identical appearance were found to be associated with a variety of diseases. The parasitic nature of traumatic diseases was probable, but unproven.

Koch began his work on traumatic infective diseases with the conviction that the most fruitful line of investigation would be a comparative one, namely, to induce septic infections in animals and see whether they would "breed true" upon successive reinoculations, controlling the experimental observations by careful microscopic examination throughout. He used for the purpose of infecting his animals putrid serum or bouillon. This, he found, contained a large variety of organisms of different sizes and shapes, which he was unable to separate from one another. He hoped that, implanted into the body of an animal, a selection might occur, and only those pathogenic for the particular species survive. His anticipations were justified, and the injection of small quantities of such materials was followed, in a number of instances, by the development of a fatal illness with the presence in the blood of one only of the many forms present in the original material. He was able to carry on the disease from one animal to another, always with the same symptoms and the presence of the same organism. Moreover, if the same material containing a variety of organisms were injected into animals of different species, one microbe flourished in the one species and another in the second, showing that a particular microbe could establish itself in one animal and not in a neighbouring species.

The animal body is, as Koch said, an excellent apparatus for pure cultivation, and he succeeded to some extent in doing what had been the stumbling block to all progress, namely, to isolate one organism from another.

The publication of these three papers raised the, hitherto, obscure physician of Wollstein to the first rank of scientific investigators, but they were merely the beginning of his scientific career. Their importance and the genius of their author were recognised by Struck, the enlightened Director of the German Health Office, who invited Koch to accept a position in that department. The chemical and hygiene laboratories attached to the department had been extensively equipped, but bacteriology was naturally unprovided for. A room was, however, found for him, and in these humble surroundings he settled down to pursue his inquiries. He was soon joined by Loeffler and Gaffky, who became his first assistants. The three worked together enthusiastically in the one room, fitting up the laboratory, inventing apparatus, and improving methods. The great problem confronting them was to find a practicable means of obtaining a pure culture outside the body. Koch accomplished this by the simple expedient of adding gelatine to the nutrient medium. The gelatine-containing medium was inoculated whilst warm, with a minute amount of the material, poured in a thin layer upon a plate and allowed to set. In this way bacterial colonies originating from individual microbes were obtained. Portions from the colonies were subsequently sown into separate tubes of broth or other fluid suitable for their growth. The discovery of this technique made advance possible.

Another line of investigation undertaken at this time, on account of its importance in the technique of bacteriology, was concerned with disinfection and sterilisation. The experiments of Koch and his pupils, made upon pure cultures of pathogenic bacteria, is the foundation upon which all later work on this subject has been built. It also led to the substitution of the more convenient steam sterilisation for dry heat.

One cannot emphasise too strongly to what a large extent Koch provided the tools of inquiry at each stage in the development of bacteriology, but he did not rest there. From 1880 onwards followed a period of extraordinary activity. In a dozen years the etiological factor of eleven important human diseases—tubercle, cholera, typhoid, diphtheria, erysipelas, tetanus, glanders, pneumonia, epidemic meningitis, influenza, and plague, as well as numerous animal diseases—was discovered by Koch and his pupils.

After the completion of his work on anthrax, Koch's individual efforts were directed, more particularly, to the discovery of the infective agent in tuberculosis, whilst diphtheria and typhoid were being investigated by his assistants, Loeffler and Gaffky. The work of Klencke and Villemin and the further experiments of Cohnheim and Salomonsen, had established that the disease tuberculosis was due to an infective agent which was capable of propagating itself in the animal body. Miliary tubercles were examined microscopically for some signs of a microbe, but for long without success. At last, by a modification in the method of staining, a fine bacillus was discovered, and its presence in the majority of preparations established. Efforts to grow the organism in pure culture at first failed, but subsequently, by infinite patience, he succeeded in growing it upon coagulated serum. Once isolated and grown upon a succession of media, the establishment of the bacillus as the etiological factor presented no difficulty, and at the Physiological Society in Berlin on March 24, 1882, Koch presented the proof that he had discovered the cause of one of the most widespread and dreaded of human diseases.

From the discovery of the cause of a disease, its prevention or cure does not necessarily follow, but in the campaign against an enemy it is of first importance to be acquainted with his nature and peculiarities. Koch interested himself, at once, in studying the life-history and methods of warfare of the tubercle bacillus. These studies were, however, interrupted. Cholera was in Egypt and threatened Europe, and the German Government organized a Commission, with Koch as leader, to proceed to Egypt to study the disease, and draw up recommendations for dealing with it, should it reach Germany. Shortly after reaching Egypt the outbreak there ceased. In the meantime, however, Koch had obtained important information leading him to suspect a particular comma-shaped bacillus as the specific cause of the disease. The material for the furtherance of his inquiry having failed in Egypt, Koch proceeded to India, where cholera is endemic, and completed his investigations. He satisfied himself that the comma bacillus was the constant companion of the cholera disease, that its abundance was commensurate with the severity of the attack, and that it penetrated beneath the epithelium in the affected part. He never succeeded in obtaining it from the healthy or diseased intestine, other than in the case of cholera. It was comparatively easy to obtain it in pure culture, and its characteristics were studied, but the completion of the evidence to convict this organism was lacking, as a true cholera process cannot be artificially produced in any of the laboratory animals. Incidentally, whilst in Egypt, he discovered amœbæ in dysentery, and the bacillus responsible for the widespread ophthalmia in that country.

As previously mentioned, at the time Koch was ordered to Egypt to investigate cholera, he was engaged in the attempt to discover some means to modify the infection by the tubercle bacillus in the animal body. Proceeding on the assumption that the tubercle bacillus exercises its pathogenic effects by means of a chemical poison, Koch investigated the action of the dead bacilli and their products upon normal animals, and also the effect of a previous injection with dead bacilli, upon a subsequent inoculation of living ones. This led to the important observation that in animals previously treated with dead bacilli, the later inoculation of living organisms occasioned an energetic local reaction, leading in some cases to their destruction.

From his experiments he was led to the conclusion that, not only could a guinea-pig be immunised against tubercle by repeated injections of the products of tubercle bacillus, but that the same tissue reaction could be stimulated, and the disease brought to a standstill, by this means. Later (1890), he published his well-known results upon tuberculin, by means of which tuberculosis could be not only diagnosed, but in the early stages might, he hoped, be cured. The possible remedy was enthusiastically welcomed by the whole world. Medical men thronged to Berlin from all parts to see the results of its application. The treatment was applied to all sorts of cases in what we now know were colossal doses. The results were disappointing, and in many cases disastrous. The premature publication of his results with tuberculin was a misfortune, and the failure of the treatment obscured for the time being the great value of Koch's work, and even exposed him to opprobrium. Koch had, however, made a great discovery, but underestimated the potency of the agent he had brought to light. Tuberculin is an invaluable diagnostic for early tuberculosis in man and animals, and is universally employed. Since 1890 it has been consistently employed by a number of physicians all over the world for the treatment of tubercle with what appear to be beneficial results, and of recent years its employment has again been resuscitated by Wright. It is now administered in much smaller doses, and with careful observance of the effect of each injection.

During the next eight years, 1891–1899, Koch was occupied with investigations into a large number of diseases of men and animals. The list includes leprosy, rinderpest, plague, surra, Texas fever, and malaria. These investigations necessitated his spending much of his time abroad. It would be difficult to adjudge just how much of the knowledge gained upon these diseases to attribute to Koch, as he was accompanied by one or more distinguished workers, as in the case of the German Plague Commission which visited India. It is very clear, however, from the published reports and papers, that the insight and experience with which he directed the inquiries materially enhanced our knowledge of the causation and means of spread of these diseases.

Koch's work upon malaria needs special mention. Whilst in tropical countries, his attention was naturally drawn to this disease. Laveran's discovery of the malarial parasite had been made, but the mechanism of the spread of the disease was unknown. Manson's discovery that filaria was inoculated by the mosquito, Theobald Smith's proof that Texas fever was transmitted by ticks, and Bruce's demonstration that the tsetse-fly disease was due to a protozoan parasite, and merely conveyed by the fly, suggested to Koch, as to others, that malaria might be transmitted by a biting insect. He was, indeed, engaged upon experiments with mosquitos, and had nearly satisfied himself that malaria was thus transmitted, when Ross published his results. Koch was, however, largely instrumental in showing that the three types of malaria were associated with three distinct parasites, and that none of these were infective for the lower animals, a result of great importance, from the point of view of malaria prophylaxis. He also cleared up the difficulty as to the reservoir of the disease in a population, the adults of which could not be found to harbour the parasite, by showing that the young children, even to the extent of 90 to 100 per cent., were infected.

In 1901 Koch reported to the British Congress on Tuberculosis the results of experiments, which he had carried on during the preceding two years in conjunction with Schutz, upon the pathogenicity of the human tubercle bacillus for domestic animals. Briefly stated, Koch's main conclusion from their experiments was that human tuberculosis differs from bovine, and cannot be transmitted to cattle. The far more important question: "Is man susceptible to bovine tuberculosis?" was then considered. No direct experimental proof of this converse proposition is possible, but from the fact that men—and particularly children—consume large quantities of bovine tubercle bacilli in milk, and yet tuberculosis of the intestine is rare, Koch concluded that man is little, if at all, susceptible to the bovine variety of the bacillus. He pointed out that the question whether man is susceptible to bovine tuberculosis at all, was not decided, but expressed the belief that infection of human beings is of so rare occurrence, that it is not necessary to take any measures against it. It was the last conclusion that caused so much consternation, as most countries were embarked in considerable expenditure with a view to minimising the chances of infection by milk and meat. Koch may have been unwise in stating his views, but he did so with the conviction that bovine tubercle is not an important source of infection, and with the earnest desire that we should not squander our energies in subordinate directions, but should concentrate them in efforts to diminish man-to-man infection through the respiratory tract.

The importance attached to a considered opinion of so distinguished an authority led to the appointment of numerous commissions of inquiry in Europe and America. Of these the work of the English Royal Commission has been the most extensive. These investigations have shown that the sharp distinction between the two varieties, though usually manifest, is not so absolute as Koch supposed, and that bacilli of the bovine type are not so uncommonly found in human infections as he was led to believe. The frank expression of opinion by Koch on this subject has been the stimulus for an enormous amount of valuable work in connection with tuberculosis throughout the civilised world, but the relative importance of infection from one another, through sputum, and from bovines through dairy produce, is still an open question, and will not be settled for many years to come.

Before closing this sketch of his life-work, it remains to add a few words upon Koch as a teacher. In 1885 he removed from the Health Department, and became a professor in the faculty of medicine and director of the new Hygiene Institute, attached to the University of Berlin. Here, with the help of his assistants, numbers of those who later became the leading bacteriologists in all countries were trained in his methods and endowed with some portion of his enthusiasm and earnestness. The admiration with which he was regarded by his pupils, and the absolute faith which he inspired, amounted in many cases to actual worship, and afford further evidence of the essential greatness of the man.

Amongst the numerous honours conferred upon him by scientific and academic bodies throughout the civilised world was the Foreign Membership of the Royal Society, to which he was elected in 1897.

There have no doubt been many discoverers as great as Koch, but it must be seldom that one has been so individually associated with the development of a science. Bacteriology has to so great an extent grown up around Koch, that the title "Father of Bacteriology" has been conferred upon him by his admiring compatriots.

C. J. M.

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